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HEREDITAS

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ABERRATIONS IN THE NUCLEOLAR CHROMOSOME OF INBRED RYE

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I. INTRODUCTION

ON evidence obtained from his investigations on intraspecific sterility in *Galeopsis*, MÜNTZING (1938, 1945) suggested that small cytological aberrations are of wide-spread occurrence in the population of auto- and allo-gamous plants. He pointed out that the possibility of both the homologues of a chromosome having the same defect, was rare, so that any small structural defect present in a single dose in a plant would usually be completed by its normal homologue, the plant itself suffering no deleterious effects. When, however, such a plant is selfed, the structural defect may become homozygous in the inbred offspring and could be partly responsible for the inbreeding depression that follows.

Structural aberrations and heterozygosity have been reported to be present in the population of some of the plant and animal species (GILES, 1940; DOBZHANSKY, 1941, *et al.*). In rye, MÜNTZING and PRAKKEN (1941) have reported the presence of various types of chromosome aberrations in population plants. They have estimated them to be as high as 6 % of the plants growing in the field.

It is not very surprising, therefore, that different structural defects have been reported to be present, now and then in inbred material, and in some cases, even in long time inbred lines (LAMM, 1936, PUTT, 1954, and REES, 1955 in rye; CLARK, 1942 in maize).

In the present paper some aberrations found in certain chromosomes in the different inbred lines of rye are described. In some cases attempts have been made to find their origin, and to see if some of the aberrations present in the inbred lines could be traced back to the population.

As most of the observations were made on a particular pair of chromosomes, in the somatic cells, ease of identification of the chromosome pair was essential. For this purpose the nucleolar chromosome with its large satellite, and submedian primary constriction, was considered ideal. This chromosome corresponds to the satellite chromosome S′ of

LEVAN (1942), chromosome No. VII of TJIO and LEVAN (1950) and chromosome No. V of LIMA-DE-FARIA (1952).

II. DEFINITIONS

The satellite chromosome has been divided into three well defined regions (Fig. 1). (1) The long arm region extending from the distal end of the long arm to the kinetochore and often described in the text by the letters L. A. (2) The short arm region »A» extending from the kinetochore to the nucleolar constriction region. (3) The short arm region »S» extending from the nucleolar constriction to the distal end of the

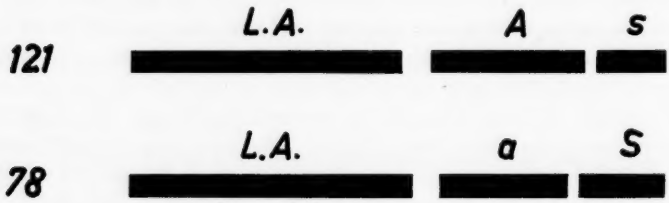


Fig. 1. Diagramatic representation of the nucleolar chromosomes typical for inbred line 121 (above) and inbred line 78 (below) and the symbols used in describing the various regions of the chromosomes, as used in the text. L. A.=long arm, A=large short arm, a=small short arm, S=large satellite, s=small satellite. The term short arm should actually include regions A+s (or a+S) but for the sake of brevity the above system has been adopted in the text. Lengths of the segments of the chromosomes are approximately proportional to measurements given in Table 3.

satellite. The nucleolar constriction region and the kinetochore region have been disregarded for the moment as they do not play an important rôle in the present investigation.

Most of the work presented in this paper deals with inbred line No. 78, inbred line No. 121 and their hybrids and the nomenclature procedures explained below strictly apply to these two lines and their hybrids.

It will be shown later that short arm region »A» and short arm region »S» (the satellite) may vary in length in the two inbred lines whereas the long arm (L. A.) remains constant. A rough method of estimating whether short arm region »A» is large or small, is to divide the length of the long arm by the length of the short arm (region »A»). Since in these two lines the long arm remains constant in length a quotient greater than two indicates a small short arm (region »A») whereas a quotient

less than two indicates a larger short arm. In order to distinguish these two types a capital »A» has been used to indicate the larger type, and a small »a» for the smaller type of the short arm.

In the same manner a capital »S» is used for a large satellite and a small »s» for a small satellite. For the sake of brevity the term »short arm» will be used in future to mean the short arm region »A», or »a», and the term »satellite» will be used for denoting the region »S», or »s»; strictly speaking, however, the term »short arm» includes both the region »A» (or »a») and region »S» (or »s»).

Owing to their small size it is more difficult to assign the correct class, to which a satellite belongs, especially in certain cases described later, where a satellite belonging to one particular type has been transferred to another type, with a different length of short arm. Generally, the satellite »S» belonging to line 78, is more than half as big as its own short arm »a» but less than half the size of the short arm »A» belonging to line 121. The small satellite »s» is less than half the size of both »A» or »a». Another criterion, used in distinguishing the two types of satellites, is the presence or absence of a large terminal knob in the satellite at the somatic prophase stage. Thus the large satellite has a large knob, associated with it; in the small satellite, however, this knob usually cannot be detected. As is well known in rye, all the seven pairs of chromosomes are characterized by large and usually terminal knobs. The number and position of the knobs have been described by TJIO and LEVAN (1950) and LIMA-DE-FARIA (1952). Similar knob-like structures have been described in other species also, such as maize and teosinte, and LONGLEY (1939) has discussed the nature of their origin. He regards them as enlarged chromomeres, heterochromatic in nature and occurring only in certain favourable positions of the chromosome. LIMA-DE-FARIA (*l. c.*) has shown that the knobs of rye are composed of an association of several large pairs of chromomeres, and hence prefers to use the term »knob formation» when describing them. Thus, he finds that the nucleolar chromosome of rye at pachytene has two pairs of large chromomeres at the nucleolar organizing body, and at least three pairs of large chromomeres at the terminal knob formation region of the satellites. For the sake of brevity the term knob has been mostly used in this paper, except in some cases where more accuracy has been needed.

Since knobs of rye show strong affinity to staining in the resting nuclei in early mitotic prophases (LEVAN, 1936; TJIO and LEVAN, 1951) they have been regarded as being heterochromatic in nature. As used here, the term may or may not include those regions showing nucleic

acid starvation, a criterion used by DARLINGTON and LA COUR (1941) and LA COUR (1951) in defining heterochromatic regions in the more stricter sense of the word. LEVAN (1936) has shown the presence of such starved regions in the terminal knob formation regions of the short arms of rye.

Finally mention must be made of the meaning of the word »control» as used in the text. A number of observations have been made on the pachytene stages in the F_1 and F_2 hybrids between lines 78 and 121. These have been compared with similar studies made on material obtained from Dr. LIMA-DE-FARIA. This material is a hybrid between Västgöta rye and an inbred line of Steel-rye and has been referred to in the text as »control» material.

III. MATERIAL AND METHODS

The inbred material described in this paper, has been kindly made available by Professor MÜNTZING from his extensive collection of inbred lines, some of which have been selfed for twenty-five years or more. These lines were originally developed from the Swedish commercial variety »Stålråg» (Steel-rye) which is a selection from the variety »Stjärnråg», and this in turn has been derived from the variety »Petkus råg».

The four main lines investigated here are the following: line 78 (1951), line 121 (1951), line 113 (1953), and line 114 (1953). Of these, lines 78 and 121 have been worked out more in detail.

The number of a line belongs to the particular year it was first examined. This number has been kept unchanged in the subsequent years, instead of giving them new numbers every year as is commonly done in this institute.

Most of the data given here have been obtained from the somatic chromosomes of the root-tip cells. In order to obtain good metaphase plates a pretreatment of the roots with oxyquinoline was found to be very helpful (TJIO and LEVAN, 1950). By this treatment, chromosomes become straightened out and are well spread, thus greatly facilitating their measurements. Root tips were cut off from seedlings germinated on moist filter paper, in petri dishes, and were immersed in oxyquinoline solution (0,03 m/l) for six hours at about room temperature. They were then stained and squashed in aceto-orcein, following the method described by TJIO and LEVAN (*l. c.*). Slides were made permanent by taking off the coverslips in 95 % alcohol, and remounting slides and

coverslips in balsam, after passing them through grades of absolute alcohol and xylol.

Sometimes a very weak solution of colchicine was used as when large numbers of divisions were required. This was necessary in certain lines where it was difficult to find divisions in sufficient numbers. Colchicine has also been used for comparing its effects with that of oxyquinoline on the different parts of the chromosome.

Measurements were taken by projecting the image of the chromosome or parts of the chromosome on to a blank sheet of paper placed at bench level. Then by means of a fine pair of dividers, lengths of the different parts of the chromosomes were taken, and measured off against a millimeter scale. In this way much time was saved, without sacrificing accuracy. For statistical treatment the millimeter was regarded as the minimum class unit, so that all readings falling, say, between 5 m. m. and 5,9 m. m. were grouped under the 5 m. m. class.

For study of meiosis, spikes were fixed in Carnoy (1 glacial acetic acid; 3 absolute alcohol) for about 3—4 hours, washed and kept overnight in 95 % alcohol, and then stored in 70 % alcohol. Pollen mother cells were teased out from the anthers and stained in aceto-carmine, following the methods of LIMA-DE-FARIA (1948, 1952). Slides were made permanent when necessary, following MCCLINTOCK's schedule.

IV. OBSERVATIONS

In Table I is given the distribution frequency of the long arm/short arm ratios of the nucleolar chromosomes of the two inbred lines 78 and 121. It will be seen that ratios have been given for two years (1951 and 1952) for each of the lines. It is clear that the two lines differ from one another not only in 1951 but also in 1952, and that this difference is significant (*cf.* Table 4). It can also be seen that there is no significant difference within each line in the two years. In Table 2 is given a variance analysis confirming the above views, *viz.* a significant difference ($P < 0{,}001$) found between the lines but no significant difference found within lines, between years.

Values obtained from population plants grown in 1952 are given in the last line of Table 1 and it can be observed that line 78 ($\bar{x} = 2{,}47$) approaches nearest the ratio of the population plants ($\bar{x} = 2{,}35$). The other line (line 121, $\bar{x} = 1{,}94$) may therefore be regarded as the more aberrant type. A closer examination revealed that the observed differences in the ratios between the two lines, were caused by differences in

TABLE 1. *Distribution frequency of the L.A./S.A.[1] ratios of the nucleolar chromosomes in the inbred lines 121 and 78 and the population.*

Year	Line	Ratio	1,5	1,6	1,7	1,8	1,9	2,0	2,1	2,2	2,3	2,4	2,5	2,6	2,7	2,8	2,9	3,0	3,1	3,2	3,3	N	$\bar{x}$
1951	121		3	4	10	25	8	26	3	3	1											83	1,92
	78						1	3	2	9	5	8	3	3	2	3	0	3	0	1		43	2,47
1952	121			5	7	5	8	12	2	1	2	1	0	1								44	1,98
	78					1	0	0	1	5	5	6	3	2	2	1	0	1				27	2,45
1952 — Pop.						2	2	4	8	9	9	2	3	4	0	7						50	2,35

Total No. of chromosomes measured = 247

[1] Short arm here (and in subsequent tables) refers to the region between the kinetochore and the secondary constriction region (*cf.* Definitions and Fig. 1).

TABLE 2. *Analysis of variance of the ratios of the arms of the nucleolar chromosomes of inbred lines 121 and 78 from the data in Table 1.*

Cause of variation	Sum of squares $s(x-\bar{x})^2$	DF.	Variance $\frac{(s(x-\bar{x})^2}{n-1}$	Quotient
Total variation	2212,508	196	—	—
Between lines	1236,865	1	1236,865	247,67*** ($P < 0,001$)
Between years	6,778	1	6,778	1° ($P \sim 0,5$)
Within population	968,865	194	4,994	—

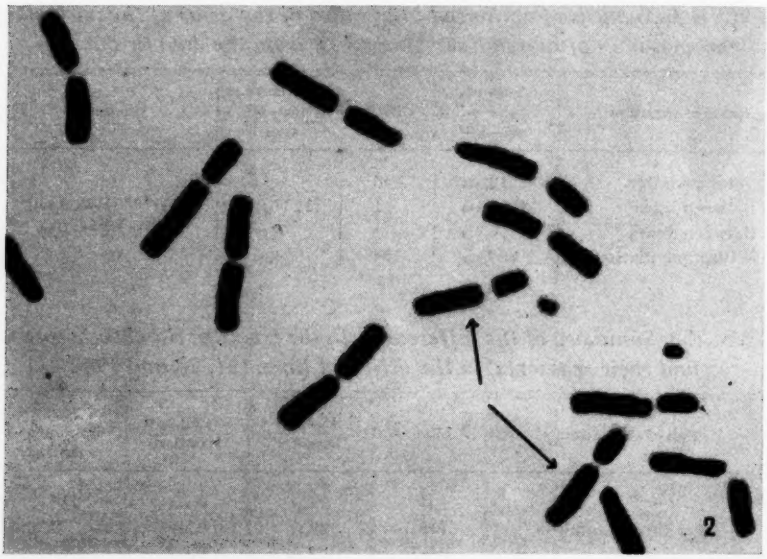
TABLE 3. *Summary of the differences, in the length of the chromosomes and their segments, in the different lines 121, 78 and Pop.*

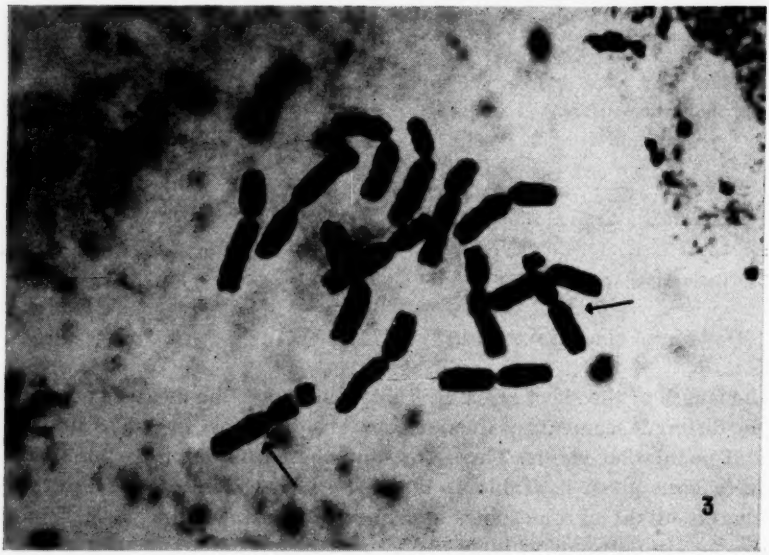
Region of chromosome	Line	Mean $\bar{x}$ length [1]	S (standard deviation)	No. of chromosomes measured
(1) Total chromosome	121	22,45	0,513	111
	78	22,24	0,369	73
	Pop.	22,44	0,578	50
(2) Long arm	121	13,04	0,212	127
	78	13,45	0,344	77
	Pop.	13,64	0,336	50
(3) Short arm (excl. sat.)	121	7,16	0,116	127
	78	5,88	0,134	77
	Pop.	6,24	0,171	50
(4) Satellite	121	3,32	0,063	111
	78	3,89	0,091	73
	Pop.	3,52	0,087	50
(5) Ratio of long arm/short arm	121	1,94	0,0029	127
	78	2,47	0,0328	70
	Pop.	2,35	0,0396	50

[1] All lengths in m. m. at $\times 2700$.

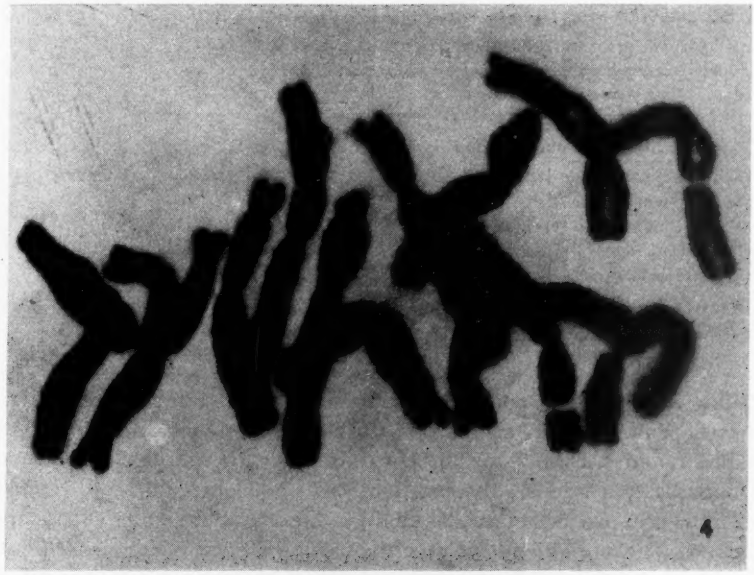
the length of the short arms. In Table 3 are given the measurements for the different segments of the nucleolar chromosomes, in line 78, line 121, and population plants. These are from the chromosomes whose ratios have been given in Table 1. It can be seen that the total (average) lengths of the chromosomes and also the (average) long arm lengths are nearly the same in lines 78, 121 and population, indicating thereby that they are constant in length in all three cases.

The short arm of line 121 is, however, significantly larger than that





Figs. 2—3. Showing the somatic metaphase chromosomes of line 121 (Fig. 2, above) and line 78 (Fig. 3, below). Nucleolar chromosomes marked with arrows. Note the relatively longer short arms (region »A») and smaller satellites (»s») in the nucleolar chromosome of line 121, as compared to similar regions in line 78. Oxyquinoline treated. (Fig. 2, *ca.* ×2200, Fig. 3, *ca.* ×1000.)



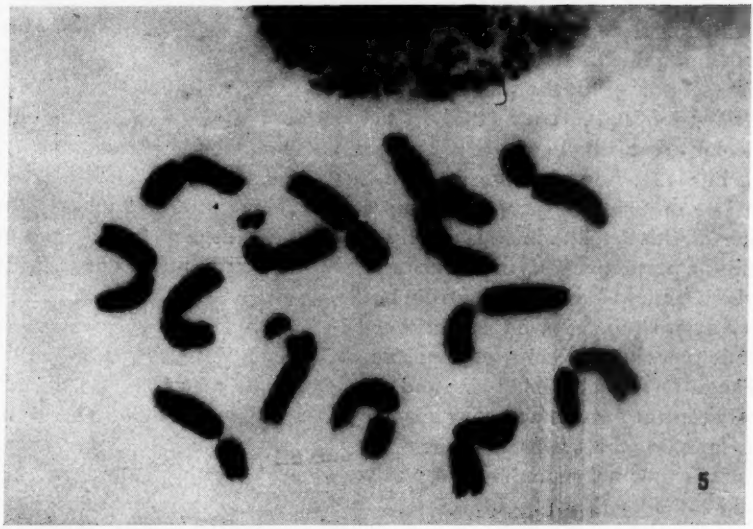


Fig. 4 (above). Somatic metaphase of an F_1 (121×78) hybrid having the chromosome type As/aS. Note the differences in length in the short arm regions and the satellites between the two homologous nucleolar chromosomes. Colchicine treated. (*ca.* ×3700.) — Fig. 5 (below). Somatic metaphase of an F_2 hybrid, of the same type as above, and showing similar differences. Oxyquinoline treated. (*ca.* ×2800.)

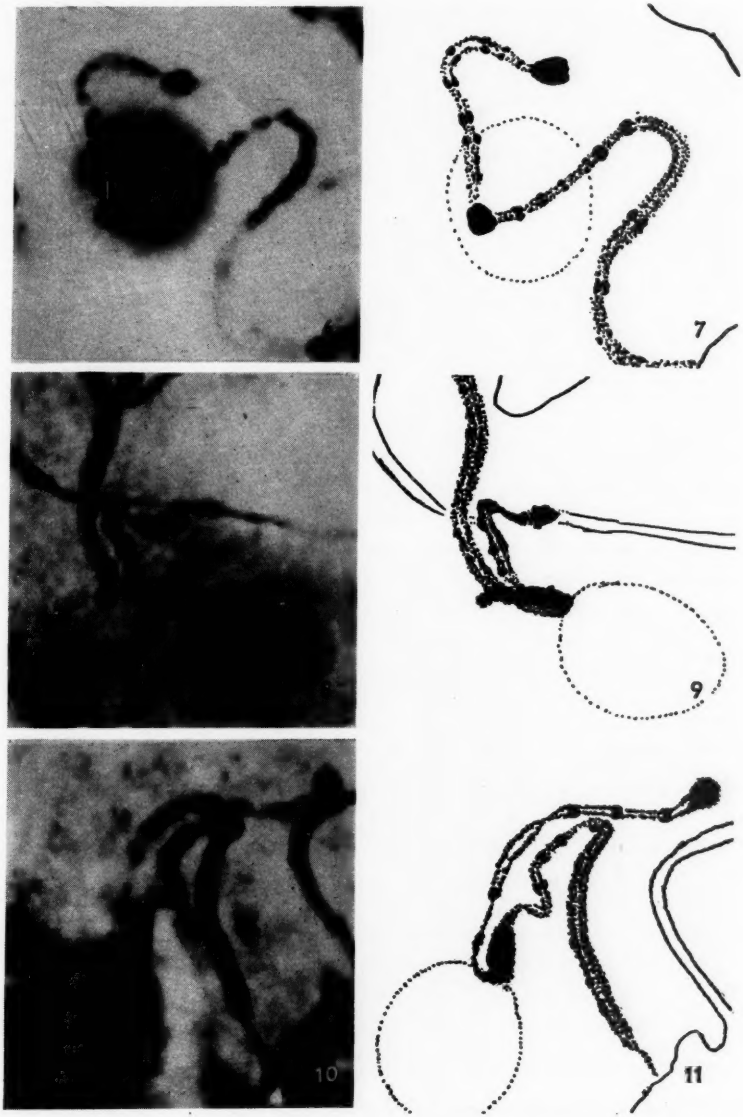
TABLE 4. *Showing the »t» values of the different chromosome segments between the different lines compared.*

Lines compared	121 *vs.* 78	121 *vs.* P	78 *vs.* P
Region of the chromosome			
(1) Total chromosome length	0,33	0,14	0,30
(2) Long arm length	1,01	1,51	0,40
(3) Short arm length (excl. sat.)	7,23***	4,40***	1,65(?)
(4) Satellite	5,06***	1,91(?)	2,98**
(5) Ratio of long arm/short arm	15,99***	8,06***	0,3

*** = highly significant, ** = significant.
(?) = doubtful values (probably significant).

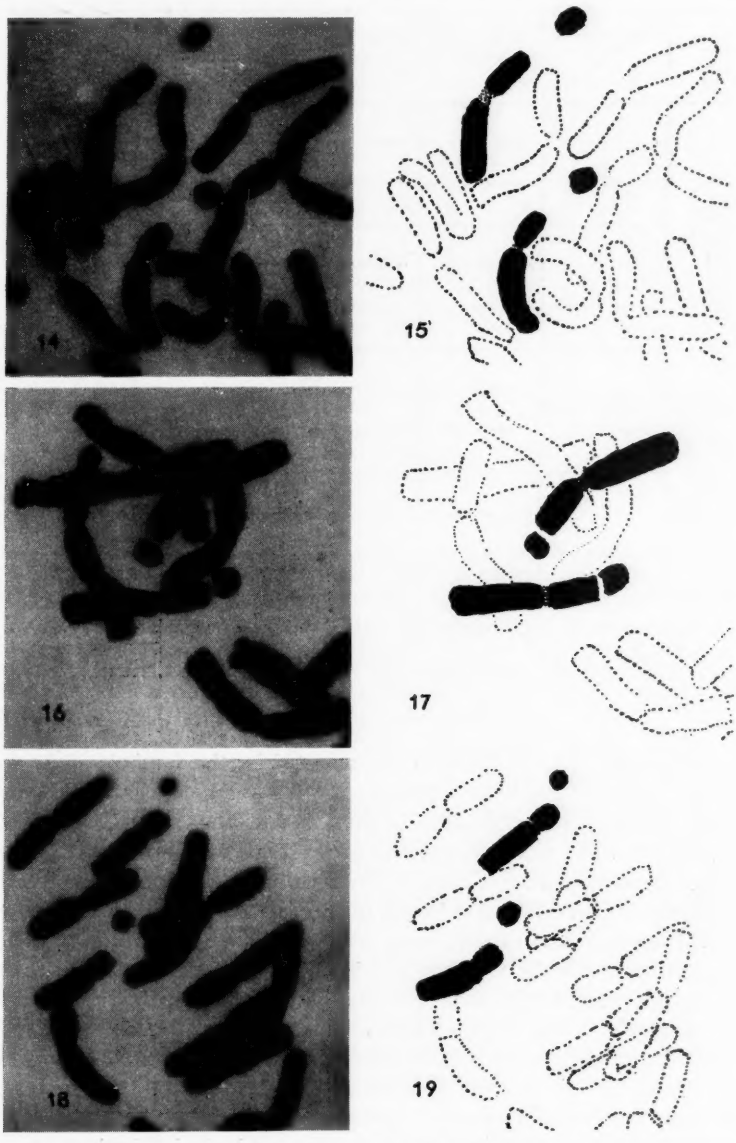
of line 78 ($P < 0{,}001$) and at the same time the satellite is smaller than that of line 78, and, again, this difference is significant ($P < 0{,}001$; Tables 3 and 4, Figs. 2 and 3). On examining the mitotic and meiotic prophases (pachytene), these differences in length can be correlated with the presence or absence of knobs in the satellites and the short arm regions. The normal nucleolar chromosome of rye has a large knob formation at the extremity of its long arm, a second terminal knob formation region at the distal end of the satellite portion of the short arm and a third intercalary knob formation, situated near the nucleolus, corresponding to the nucleolar organizing body of maize, as described by McCLINTOCK (1934). Usually the satellite knob and the nucleolar organizing body are of approximately equal size (Figs. 6 and 7). In line 121, however, the satellite knob is distinctly smaller in size, visible only in the pachytene, but invisible in mitotic prophases. In line 78, this satellite knob is clearly visible both in the pachytene and in mitotic prophase. This difference can best be seen in mitosis of hybrids which contain chromosomes from both of its parents in each cell. Fig. 12 is a photograph of such a hybrid cell in the prophase stage containing both types of nucleolar chromosomes, belonging to lines 78 and 121. The differences in the knobs, and disparity in length of the short arms, between the two homologous chromosomes can be readily observed. In metaphase, the knobs disappear and the difference between the two lines is now only manifested by differences in length of the short arm and satellites (Figs. 2—5).

In pachytene the differences in the knob sizes are clearly apparent. Figs. 6—11 are photographs and their respective drawing interpretations of a control plant (Figs. 6 and 7), an F_1 hybrid (Figs. 8 and 9) whose



Figs. 6—11. Microphotographs and respective drawings of the nucleolar chromosomes in the pachytene stages. Figs. 6—7 control plant; Figs. 8—9, F_1 (As/aS type) hybrid; and Figs. 10—11, F_2 (AS/as type) hybrid. Note the presence of a much larger knob in the nucleolar organizing region of the hybrids as compared with the control. Aceto-carmine. (*ca.* ×3500.)

Fig. 12 (above). Mitotic prophase of an F_2 hybrid (As/aS type). Showing the presence of the large knob in the satellite marked by arrow and capital »S» belonging to line 78 type of chromosome but which cannot be seen in the other satellite marked by arrow and small »s» belonging to chromosome of line 121 type. Arrows marked with capital »A» and small »a» refer respectively to the large and small short arms of the nucleolar chromosomes. Oxyquinoline treated. (*ca.* ×2800.) — Fig. 13 (below). Somatic metaphase chromosomes of line 113, characterized by relatively short long arms. Oxyquinoline treated.



Figs. 14—19. Microphotographs and respective drawings of the somatic chromosomes of some F_2 plants obtained from selfing an F_1 (121×78) hybrid. Figs. 14—15 AS/aS type; Figs. 16—17 As/AS type; and Figs. 18—19 aS/as type. Oxyquinoline treated. (*ca.* ×2800.)

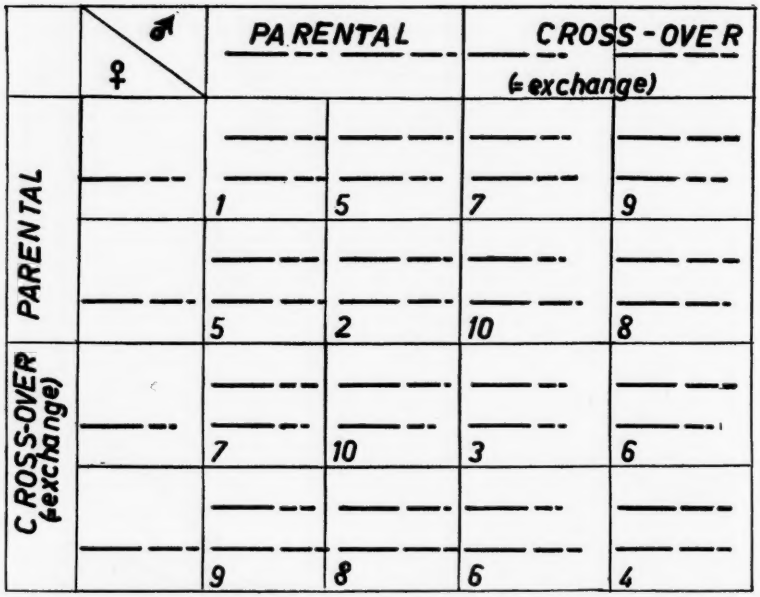


Fig. 20. Scheme showing the different types of chromosome combinations possible in the F_2 plants as expected on selfing an F_1 hybrid with heteromorphic (nucleolar) chromosome pair. Note the new homomorphic types No. 3 and No. 4. See text for further explanation.

nucleolar chromosomes may be represented by the symbols aS/As, and an F_2 hybrid (Figs. 10 and 11), having the symbols as/AS. These two types of hybrid are represented in Figs. 20 and 21 — as case 5 and case 6, respectively. The much larger size of the knob in the nucleolar organizing region compared to the satellite region is clear in both the hybrids and has also been found to be present in line 121, where this difference is still greater, due to the absence of the terminal knob of line 78, which is not the case in the hybrids. The knobs in the control plants are also more uniform in size and the nucleolar organizing body is smaller (Figs. 6—7; compare also Figs. 20—24, LIMA-DE-FARIA, 1952). (The control material have been obtained from fixations made by LIMA-DE-FARIA, and are crosses from Östgöta and inbred Stålråg; they have been used here for purposes of comparison.)

The large size of the nucleolar organizing body in the hybrids and line 121 is probably due to duplication, or presence of extra (and

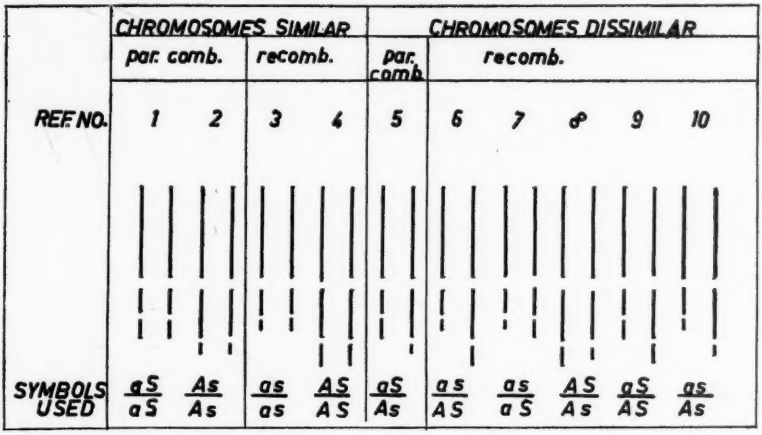


Fig. 21. Showing the ten different types of chromosome arrangements expected in F_2 plants, and the symbols used in describing them. The numbers refer to the types shown in Fig. 20.

proximal) chromomeric bodies there being present, at least three chromomeric bodies in the hybrid, instead of the normal number two, as reported by LIMA-DE-FARIA (1952). At the same time the small size of the satellites in line 121 seems to be due to the absence of at least a part of the terminal knob. Whether these two phenomena are interrelated and how they may have arisen, will be discussed later.

Fig. 20 is a scheme where the expected segregation for the different types of chromosome combinations is shown and is based on the assumption that crossing over takes place on or near the secondary constriction region, of the nucleolar chromosome. Without further genetical evidences the validity of this hypothesis cannot be fully tested, though there seems to be a general agreement on the types found and the types expected amongst the F_2 progenies.

An explanation of the frequent occurrence of crossing over in this sector of the chromosome may be that the nucleolar constriction region is a more fragile area sensitive to stress and easily ruptured by those forces acting in the early prophase stages which, according to DARLINGTON (1937), are responsible for the general phenomena of crossing over.

Returning to Fig. 20, it will be seen that an F_1 hybrid having a heteromorphic pair of chromosomes can, on selfing, give rise to ten different types of chromosome arrangement in the zygote, due to the

random union of cross over and non-cross over gametes. These ten types have been shown in Fig. 21. Nos. 1—4 are zygotes with homomorphic (or similar) chromosomes, of which Nos. 1 and 2 represent the two original parental types (*cf.* Figs. 1 and 2); Nos. 3 and 4 are recombinants, and represent new synthetic types. Nos. 5—9 are the various cases of heteromorphic types of recombinants expected, of which No. 5 only has the parental combinations present, as found in F_1 hybrids.

The different types have been classified as follows:

Case 1. Short arms small and similar, satellites large and similar.

Case 2. Short arms large and similar, satellites small and similar.

Case 3. Short arms small and similar, satellites small and similar.

Case 4. Short arms large and similar, satellites large and similar.

Cases 5 and 6. Short arms and satellites both dissimilar (Figs. 4, 5 and 12).

Case 7. Short arms small and similar, satellites dissimilar (Figs. 18 and 19).

Case 8. Short arms large and similar, satellites dissimilar (Figs. 16 and 17).

Case 9. Short arms dissimilar, satellites large and similar (Figs. 14 and 15).

Case 10. Short arms dissimilar, satellites small and similar.

The symbols used in denoting the different types are given below the drawings of each chromosome pair, in Fig. 21. The methods used in giving the symbols have already been explained before.

In an actual experiment, kernels from selfed spikes of two different F_1 hybrid plants (Pl. No. 11—6, and Pl. No. 7) were germinated in petri

TABLE 5. *Frequency of different F_2 types of plants actually found on selfing two F_1 plants (Pl. No. 11—6 and Pl. No. 7).*

	F_2 segregants											
Class (= Ref. No.)[1]	1	2	3	4	5	6	7	8	9	10	Lethals	Total
Chromosome types[1]	aS/aS	As/As	as/as	AS/AS	aS/As	as/AS	as/aS	AS/As	aS/AS	as/As		
Pl. No. 11-6	3	2	0	2	12	9	5	2	4	0	3	42
Pl. No. 7	2	1	0	1	6	2	0	2	4	1	1	20
Total	5	3	0	3	18	11	5	4	8	1	4	62

[1] *Cf.* Figs. 20 and 21.

dishes and their chromosome types determined in the usual way. Table 5 gives a summary of the various types of plants found in the F_2 generation, from 58 kernels (excluding 4 lethals) that could actually be determined, from the two spikes, having an actual total of 93 (71+22) kernels. It is not certain that the figures given in Table 5 represent the correct proportion of the different types recovered, since some errors are to be expected from such factors as mistakes in identification, contamination by foreign pollen grains, and possible occurrences of chiasmata at other points than the nucleolar constriction regions. Hence, it may be too early to draw any conclusions from the numbers expected and the numbers found and so no comparisons have been attempted. Nevertheless, it is interesting to note that all the possible combinations (except case 3, Fig. 20, having the symbol as/as) have been recovered in the F_2, thus providing some basis for the hypothesis of crossing over occurring in the nucleolar constriction region.

Figs. 5, 12, and 14—19 are examples of some of the types found in the F_2. According to the symbols used in Fig. 21, chromosomes in Figs. 5 and 12 can be represented by the letters aS/As — obviously case or type 5 (Fig. 21). Similarly, Figs. 14—15 can be represented by the letters AS/aS (case 9), Figs. 16—17 by the letters AS/As (case 8), and Figs. 18—19 by the letters aS/as (case 7).

The most interesting plants obtained from the F_2 progenies were the new synthetic plants having homomorphic chromosomes, represented by the symbols AS/AS (case 4, Fig. 21). These plants are regarded as having a small duplicated knob in the large chromomeres near the nucleolar organizing body — a character derived from line 121 — in addition to the presence of the normal knob in the satellite region.

Only three such plants were found in the F_2 and though all of them show a less vigorous growth habit than their sister plants, the number is too low to allow any conclusion at this stage. In this connection it is also interesting to observe that no plants having the symbols as/as (case 3, Fig. 21) and consequently representing a deficiency type, have yet been recovered in the F_2.

At present the most instructive lesson that can be drawn from the results of the above experiment, is that a definite mechanism has been demonstrated, on the manner in which plants having heteromorphic pairs of chromosomes can give rise, on selfing, to plants having homomorphic pairs of chromosomes, the combination of some of which may be of an entirely new type.

The importance of this in explaining the mode of origin of homo-

TABLE 6. *Distribution frequency of L. A./S. A. ratios of the nucleolar chromosomes of the two inbred lines 113 and 114 and their hybrid.*

Year	Line	Ratio	1,5	1,6	1,7	1,8	1,9	2,0	2,1	2,2	2,3	2,4	2,5	2,6	2,7	2,8	N	$\bar{x}$
1953	113		1	4	3	6	9	8	2	1							34	1,9
1953	114						1	2	6	5	11	2	2	2	1		32	2,3
1954	113×114	(113)=		1	1	2	3	2	2	1	1						13	
		(114)=						1	1	1	1	4	1	3	1		13	
	Total (113×114)			1	1	2	3	3	3	2	2	4	1	3	1		26	

zygous aberrant lines, like line 121, from a heterozygous population plant containing such an aberration in one of its nucleolar chromosomes, need hardly be stressed.

The nucleolar chromosomes of a few more inbred lines have been examined to see if any aberrations were to be found in them. Table 6 gives the distribution frequency of the L. A./S. A. ratio of two inbred lines, No. 113 and No. 114 of the year 1953. A significant difference has been found in the ratios, between these two lines, and line 113 having a ratio of 1,9, has been regarded as the more aberrant type. In the F_1 (1954) hybrid (between these two lines) this difference can be seen in each of the mitotic cells where divisions have been found. This is shown in the last three rows of Table 6, where the values for the thirteen pairs of homologous chromosomes are given chosen from thirteen complete cells at random. It can be seen that each pair contains one homologue

TABLE 7. *Summary of data obtained for the lengths of the chromosomes and their segments in lines 113 and 114.* ($\bar{x}$=*mean*, m=*standard deviation*, N=*total numbers examined.*)

Region of chromosome	Line 113			Line 114			»t«	P
	$\bar{x}$ [1]	m	N	$\bar{x}$ [1]	m	N		
(1) Total chromosome length	25,47	0,669	34	26,97	0,783	32	1,19	$P < 0,2$
(2) Long arm length	14,35	0,364	34	16,00	0,447	32	7,98***	$P < 0,001$
(3) Short arm (without the satellite region)	8,15	0,226	34	7,38	0,295	32	2,07*	$P < 0,05$
(4) Satellite length	4,11	0,13	34	4,59	0,017	32	28***	$P < 0,001$
(5) Ratio L. A./S. A.	1,9	0,029	34	2,3	0,034	32	12***	$P < 0,001$

[1] All lengths in m. m., at × 3500.

TABLE 8. *Distribution frequency of the L. A./S. A. ratios of the nucleolar chromosomes of inbred lines 2, 5, (2×5), 75 and 76.*

Year	Line	Ratio	1,4	1,5	1,6	1,7	1,8	1,9	2,0	2,1	2,2	2,3	2,4	2,5	2,6	2,7	2,8	2,9	3,0	3,1	3,2	3,3	3,4	3,5	N	x̄
1953	2		1	0	1	2	8	19	9	3	0	0	0	0	1										44	1,98
1953	5								1	7	3	7	7	8	2	0	2	2	2	0	0	1			42	2,46
1954	2×5							1	2	4	0	1	1	1	0	1	1								12	2,22
								4	4	2	0	1	0	0	1	0	0								12	2,21
	Total	(2×5)						5	6	6	0	2	1	1	1	1	1								24	
1953	75								1	0	2	5	3	5	6	4	8	0	1	0	1	4			40	2,70
1953	76								1	0	8	1	2	2	2	3	1	0	4	0	0	2	0	1	27	2,64

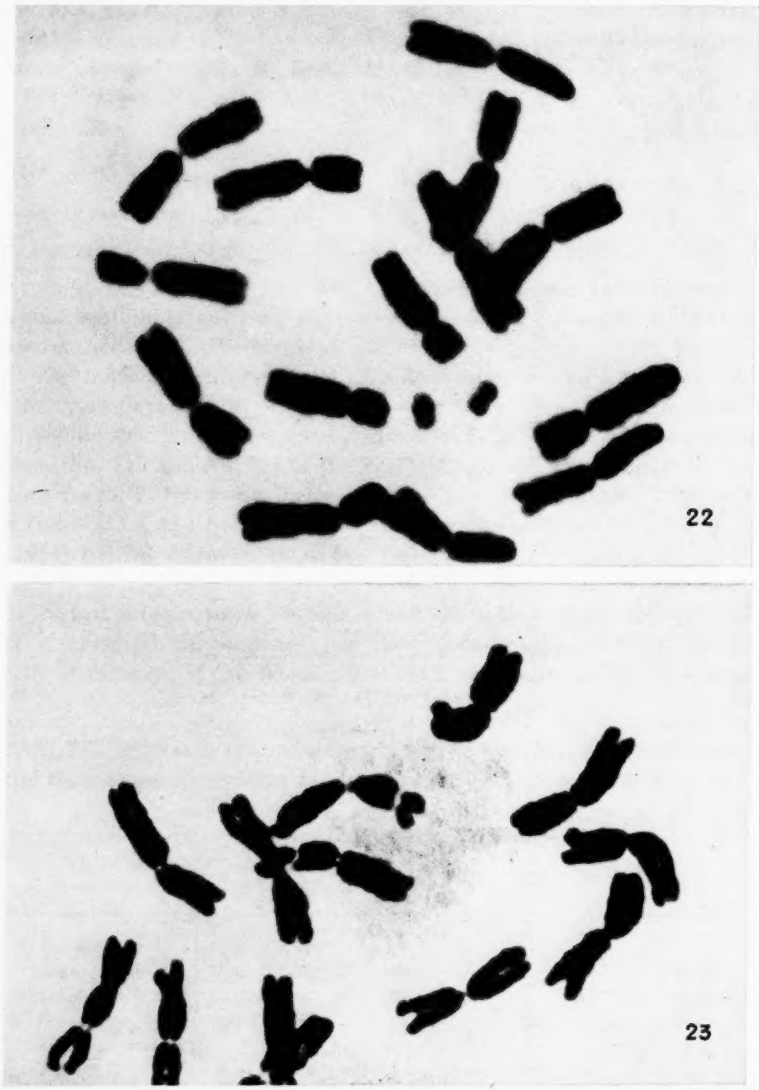


Fig. 22 (above). Somatic metaphase of line 5. Oxyquinoline treated. (*ca.* ×3800.) — Fig. 23 (below). Somatic metaphase of line 2. Oxyquinoline treated. (*ca.* ×2400.)

belonging to the 113 type and the other homologue belonging to the 114 type.

A closer analysis has revealed that the main reason for this deviation in line 113 is caused by a shortening of the long arm of the chromosome. In addition there seems to be a slight but significant difference in the satellite size (Table 7 and Fig. 13). There may be a slight difference in the short arm, but this is probably a negligible value. The pachytene configuration of the hybrid has not yet been observed, so the exact nature of the difference between the two lines is still a matter of speculation. Observations on the mitotic prophases have failed to reveal any knob differences.

In Table 8 is given the L. A./S. A. ratios of several other inbred lines. Lines 2 and 5 (of 1953) showed a striking difference at first (Figs. 22—23) in the homozygous condition but on crossing lines (2×5) this difference in the F_1 plants for unknown reasons disappeared to a large extent. A difference in the size of the terminal knob of the long arm may sometimes be seen in the 2×5 hybrids in mitotic prophase. For the sake of comparison, details of two other lines are given. These are line Nos. 75 and 76 of the year 1953 (Table 8). In these cases some differences in the values of the ratios from the population have been obtained but no further work was carried on with them.

It will be observed that no direct comparison has been made between values obtained for lines 121 and 78 and those appearing later. The reason for this is that a different drawing apparatus has been used in the latter case. Where only ratios are concerned, however, such comparison will probably not introduce any serious errors.

V. DISCUSSION

1. The validity of the techniques used and results obtained

The general accuracy of the techniques used in detecting small structural aberrations in the chromosomes can be judged from the good agreement obtained between the values calculated from measurements using this method and the photographs of chromosomes of the corresponding lines, as reproduced in this paper.

If, however, one looks at the distribution curves of the measurements made (*e. g.*, Tables 1, 6, and 8) one at once notices the very wide and spread-out nature of these curves. There may be several reasons for these rather spread-out distribution curves, in addition to those due to the

rather common fault of errors in measurements and bad squashes. Probably the main cause is the difficulty in correctly identifying the exact metaphase stage in the mitotic cycle, especially after pretreatment with a chemical like oxyquinoline. Thus, the measurements given in the above tables actually represent measurements of chromosomes from early metaphase to early anaphase (*viz.* at c-anaphase). A logical conclusion of such an explanation is that all the regions of the chromosome do not contract at the same rate in the various stages of the division cycle, from prophase to metaphase. JAPHA (1939) in *Oenothera* and BROWN (1949) in *Solanum* have demonstrated the presence of differential contraction of the euchromatic and heterochromatic regions (defined as achromatic and chromatic regions by BROWN) in meiotic and mitotic division cycle. The heterochromatic regions contract less than the euchromatic region, and both of these types are present in the rye chromosome. Other explanations for such variations in measurements are (1) Presence of local differences from cell to cell. (2) Unequal diffusion of oxyquinoline in the different parts of the root. The probability that the chromosomes of different lines may react differentially to oxyquinoline is negligible at least in lines 121, 78, 113, and 114. These differences have been found in untreated material of the above lines and also in cells of their roots treated with colchicine solutions (*cf.* Figs. 4 and 5). However, to make more certain whether an observed difference is genuine or not, the particular line in question is usually crossed to another line having a more normal type of chromosome (at any rate distinct from the first line). If the aberration could be detected in the hybrid cell, it is held that a definite structural difference is present. Further observation would then be made in the pachytene of the hybrid to get a more definite idea of what had occurred.

It was found that only in line 2 was there a discrepancy of behaviour of the chromosome as observed in the pure line and in the hybrid, 2×5 (Table 8, rows 1—4). It is difficult to assign any reason why a difference present in line 2 should disappear on hybridization. It may be that the chromosomes of line 2 were rather labile and under pressure of squashing the chromosomes may have become excessively elongated, resulting in exaggerated measurements. In the F_1 this fragility evidently disappeared. Since lines 2 and 5 are sister lines, a genetical explanation is not excluded here. In the cross 113×114, the differences in the chromosomes have been retained in the hybrid cells as well as in the cross 121×78. No pachytene observations have yet been made in the 2×5 and 113×114 crosses. It may be pointed out here also that line 113 type of

chromosome has been obtained (in the heterozygous condition) in a population plant and thus may have originated from the population.

2. Lines 78 and 121; origin of aberration in line 121

Though a more detailed description has been given elsewhere regarding the difference between lines 78 and 121, a brief review will be given here before the cause and nature of these differences are discussed.

Line 121 differs from line 78 in two regions.

(a) The satellite of line 121 has a dimunitive knob, probably due to a deficiency and observable only in the pachytene. As a result of this deficiency the satellite of line 121 is seen to be smaller at metaphase stage in mitosis than that of line 78, which has retained its terminal satellite knob.

(b) At the same time the short arm region (*i. e.*, the region from the kinetochore to the nucleolar constriction region) of line 121 has a much larger heterochromatic body as seen in the pachytene, containing more than the two large chromomere pairs, usual in normal plants. It is due to the presence of this large heterochromatic body that the short arm of line 121 is seen to be longer at the mitotic metaphase, than the corresponding region in line 78, or in the population.

Though a completely satisfactory explanation regarding the origin of the large heterochromatic body in the nucleolar organizing region of line 121 cannot be given at this stage, a few tentative hypotheses have been given below.

(1) The large knob in the short arm region (and perhaps as well the diminutive satellite) may have been derived as the result of a past hybridization with some related strains or species possessing similar chromosome characteristics.

Such heteromorphic chromosomes, having one of their homologues identical in morphological characters, to the chromosomes belonging to line 121, have been found in several plants of the population (»Steel--rye») in different years. By selfing such plants, lines having these chromosomes in the homozygote condition could easily have been developed. This will be discussed more fully later.

In teosinte (*Euchlaena mexicana* SCHRAD.), LONGLEY (1937) described the presence of intercalary knobs in some of the varieties and regarded them as originating from maize through contamination. Similarly, MANGELSDORF and REEVES (1939) and REEVES (1944) regarded the presence of knobs in maize as indicating the presence of *Tripsacum* germplasm. It is interesting to note also that LIMA-DE-FARIA (1952) has

reported the presence of diminutive knob formation in the terminal satellite region, in a Turkish variety of rye, though in some cells he found chromomere pairs of average size.

The presence of the two aberrations (*viz.* size diminution of the satellite knob and size increase of the nucleolar organizing body) may be regarded (in line 121) as the end result of a single phenomenon or as the result of two separate and independent phenomena.

(2) They can be regarded as due to »stickiness» between the heterochromatic knobs of the different chromosomes, which due to unequal separation has resulted in knobs of different sizes. Such stickiness is known to be of common occurrence in heterochromatic regions such as knobs of teosinte (LONGLEY, 1937). LIMA-DE-FARIA (unpublished) has observed interknob connections in rye.

(3) The chromosome type belonging to 121 could also have arisen by the translocation of a piece of the satellite knob to the nucleolar organizing region. The mechanism for origin in this way seems to be rather complicated and involves at least three breaks, two in the satellite knob formation region and one in the proximal end of the nucleolar organizing region. One end of the translocated portion of the region between the two breaks in the satellite, could then reunite with the proximal region of the short arm, and the other end with the part containing the nucleolar organizing region. The two remaining portions of the satellite would reunite, giving rise to a diminutive knob region containing a small deficiency. In hybrids with line 78, no complicated loops or other configurations have been found to support this and similar theories positively, though such loops are not always formed in cases of small deficiencies, duplications and inversions. This is especially so when the forces of pairing are strong enough to cause non-homologous pairing (RHOADES and MCCLINTOCK, 1935).

(4) Difficulties in separation of bivalents have been observed in population rye by MÜNTZING and PRAKKEN (1941). Stretching and final breaking of such bivalents at first anaphase separation, may result in the production of deficiency duplication types of chromatids, depending on the position of the rupture and subsequent fusion, if any. Such interchromatid fusion could again cause further breakages to occur at anaphase II.

(5) MCCLINTOCK (1941) has desribed instances where forces of terminalization have been responsible for rupture of chromatids in the nucleolar organizing region. Thus, when terminalization of a chiasma formed between the kinetochore and nucleolar organizing region, is

obstructed by the presence of the large nucleolus, the forces of terminalization in some cases are strong enough to rupture the chromatids near the nucleolus. Two by two fusions of the chromatids then occur and at anaphase a bridge-like configuration is found. Depending on the position of the break, various types of duplication and deficiency could be formed.

How far such a phenomenon could occur in rye is difficult to say. The nucleolus in most cases examined, lies on one side of the nucleolar pair of chromosomes at diplotene and diakinesis stages and not between the nucleolar organizing region and the satellite as is sometimes seen in maize. (These two regions in maize are connected by the satellite thread.)

Although bridges have been seen in meiotic anaphase in rye, it has not always been possible to say whether the chromosomes concerned were the nucleolar ones or not.

(6) It has been assumed so far that the knob of the satellite in rye is terminal in position. If, however, one regards it as being situated in the proximal portion of the satellite, then the origin of the large knob in the nucleolar constriction region could be regarded simply as a result of a fusion of this proximal portion, containing the knobs with the distal end of the short arm. This would explain the presence of the large knob seen in line 121, and its apparent disappearance in the satellite. NAWASCHIN (1927) has reported such cases in various interspecific hybrids. In rye, however, evidences from pachytene and mitotic prophases seem to indicate that the satellite knob is terminal in position.

3. The pairing of pachytene chromosomes of 78×121 hybrids

A glance at the pachytene configurations of the hybrids (Figs. 8—11) will show that a fairly regular pairing occurs between the two types of chromosomes involved. At first this seems to be rather surprising, but a closer examination will reveal the presence of rather sharp twists or bends occurring at the nucleolar organizing region. This is due to the presence of extra knobs in one of the chromosome pairs, which is absent in the other. LONGLEY (1939) has reported clear cases of similar bends and twists occurring in paired chromosomes heterozygous for knobs. A further observation made by LONGLEY in the same paper having important bearings on the present problem, was the perfect pairing found by him in chromosome VI of the maize×teosinte hybrids, in spite of the shorter chromosome of teosinte. In one hybrid he reported that the teosinte chromosome even exceeded the length of the maize chromosome, thus, »indicating that the chromosomes have the ability to

extend when necessary to bring about gene to gene pairing». This agrees rather well with the observations given in the present paper, namely, the occurrence of rather close pairing in the short arm and satellite regions belonging to the chromosomes of the two lines, notwithstanding the differences in their lengths.

The difference in size becomes apparent in the later stages of meiosis (diplotene and diakinesis) and in somatic metaphase and, as mentioned before, is probably due to a differential contraction in euchromatic and heterochromatic regions of the chromosomes (BROWN, *l. c.*). Since in line 121 the short arms have an excess of heterochromatic region, they would contract less and would thus appear larger in size in comparison to the short arms of line 78. Similarly, line 78 possesses larger satellites on account of the presence of a greater (and probably normal) amount of heterochromatic knobs. This, however, is lacking in case of the satellites of line 121, and hence their smaller size. A positive relation between the presence of knobs and increase in chromosome length has also been observed by LONGLEY (1941).

VI. CONCLUDING REMARKS

As has been pointed out in the introduction, the primary object in undertaking this research was to see if any of the aberrations present in the nucleolar chromosomes of population plants could be traced down to some of the inbred lines examined; and if they were found to be present, then whether any deleterious and other effects could be attributed to them, especially in the homozygous conditions. So far as the first part of the problem is concerned, the conclusion is that the aberrations found in the nucleolar chromosomes of line 113 and line 121, have both undoubtedly originated from the population, since both these types of aberration have been found to be present in the heterozygous condition, in some of the population plants examined. Moreover, as far as line 121 (and line 78) is concerned, a clear demonstration has been given, whereby a heterozygous plant having a 121-type chromosome present in one of its complement and a 78-type chromosome in the other (and approximately equivalent to a heterozygous type of population plant) could on selfing produce different plants homozygous for either the two types of chromosomes. It has also been shown that plants with new types of homozygous chromosome combination (*i. e.*, AS/AS and as/as; Figs. 20 and 21) can arise by chance combination of some of the cross over types of gametes (Fig. 20), resulting in the creation of new

sublines. The survival of these sublines will depend on the types of aberrations present and on chance selection. It is clear that propagation of all the lines originating even from a single plant becomes impracticable, with time, and some sort of selection will have to be made. This will inevitably lead to extinction of some sublines and retention of others.

The answer to the second problem, *viz.* whether such aberrations as are present in line 121, could be held responsible for any decrease in vigour, is not yet known. The chief reason is the difficulty in conducting suitable experiments, which could clearly show phenotypic effects of such small cytological changes. The fact that most of the chromosome variations described in lines 78 and 121 and their F_1 and F_2 hybrids involve heterochromatic regions, complicate matters to a certain extent. It is quite possible that these small structural differences owing to their heterochromatic nature, will at the most have rather slight phenotypic effects, and which would be rather difficult to detect.

The fact that only rather weak genetical changes could be found even in the presence of rather high numbers of (heterochromatic) B chromosomes in rye and *Festuca pratensis* (MÜNTZING, 1954) tend to support this view.

BROWN (1949) and MANGELSDORF and CAMERON (1942) have, however, found correlation between high knob numbers of different varieties of maize examined, and such characters as high row numbers (of kernels), denting absence of husk leaves and presence of many seminal roots.

In rye there is some indication that the new synthetic type AS/AS may have a somewhat poorer growth as compared to its sister plants. However, the number of plants studied are too few to make any definite statement.

Perhaps the problem has to be approached from a different angle. LESLEY (1938) has shown that a relation exists between the size of the satellite and size of the nucleolus. A preliminary examination in rye also seems to indicate a similar relationship. How far this influences plant growth in rye remains to be seen. RESENDE (1938) has summarized some of the phenotypic effects of satellite sizes, as reported by various authors working on different plants. The evidences are conflicting and mostly negative though in one or two cases, *i. e., Crepis dioscoridis*, investigated by M. NAWASCHIN (1926) and MEDWEDEWA (1929), and *Galtonia candicans* (S. NAWASCHIN, 1927) effects have been reported. LESLEY and LESLEY (1935) have also given a summary about the effects of different types of satellites on various plants reported, though their own work in tomato has given negative results.

SUMMARY

(1) After pretreatment with oxyquinoline, it was possible to detect small cytological aberrations in the mitotic chromosomes of different lines of inbred rye. Thus the long arm/short arm (minus the satellite region) ratios of the nucleolar chromosomes of some of the inbred lines showed significant differences from one another, or from the population.

(2) A closer inspection revealed that the deviation in the L. A./S. A. ratio for one of the lines (line 113) was due to a shortening of the long arm (Tables 6 and 7; Fig. 13). In the other line the deviation was caused by a lengthening of the short arm region lying between the kinetochore and the nucleolar constriction region. At the same time (in line 121) there was a diminution in the size of the satellite (Fig. 2; Tables 1 and 3). It has been shown that this increase or decrease in lengths of the short arm and satellite regions of line 121 could be correlated with the presence or absence of heterochromatic knobs in the corresponding segments of the chromosomes.

(3) On selfing heteromorphic F_1 hybrids produced by crossing plants of line 78 (having small short arms and large satellites) with those of line 121 (having large short arms and small satellites) plants with different types of homomorphic and heteromorphic chromosome combinations segregated out in the F_2, as a result of random union between new types of ♂♂ and ♀♀ gametes formed by crossing over, in the meiosis of the F_1 plants, preceding microspore and megaspore formation (Fig. 20). Some of these plants were homomorphic and had the parental types of chromosomes (*i. e.*, lines 78 and 121) while others showed new and interesting combinations (Fig. 21). From these, three plants were recovered having a new type of homomorphic chromosome combination whose short arms resembled those of line 121 (*i. e.*, large) and whose satellite resembled those of line 78 (*i. e.*, large). These plants have been regarded as having duplications present in the heterochromatic knob formation region of the satellite as well as in the nucleolar organizing body of the short arm and appear to have a sligthly depressed growth when compared to other F_2 plants.

(4) It is suggested that lines 113 and 121 having homomorphic aberrant chromosomes in the diploid complement, have originated from the population after inbreeding, and following a similar mechanism as has been described above. An evidence for this is that heterozygous plants having one chromosome normal and the other resembling line 121 type or line 113 type have been recovered from populations grown in different years.

(5) What influence such chromosomal aberrations may have on growth behaviour of plants has been discussed.

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SELF-INCOMPATIBILITY IN RYE

I. GENETIC CONTROL IN THE DIPLOID

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I. INTRODUCTION

IN homomorphic self-incompatible Angiosperms the incompatibility reaction between pollen and style resulting in inability of otherwise functionable pollen to perform fertilization is a positive inhibitory reaction between like specificities in pollen and style formed by oppositional alleles belonging to a multiallelic locus. These gene products may be produced even at the sporophytic level or, alternatively, only at the gametophytic level after the meiotic divisions. Taking the male gametophyte, in other words, its reaction pattern is conditioned, either completely by its own genotype, or independent of that genotype, by the genotype of the pollen generator, the sporophyte. In the former case the pollen control may be termed gametophytic, in the latter case, sporophytic.

In the case of one oppositional locus in a diploid species, the sporophyte will carry two alleles, the gametophyte only one. When pollen control is sporophytic, therefore, the oppositional alleles at their active stage will regularly occur in pairs; but singly at the gametophytic pollen control. Selection for the ability of different oppositional alleles to work efficiently in pairs on the male side will thus be possible only in the case of sporophytic pollen control.

After chromosome doubling the pollen will be diploid and thus carry two oppositional alleles. When pollen control is gametophytic, this will constitute an unbalanced novelty, and competitive interaction between different alleles in the pollen will often cause a breakdown of the self-incompatibility mechanism (LEWIS, 1943, 1947, 1949; BREWBAKER, 1954). The self-incompatible diploid in that way may give rise to a self-compatible tetraploid. On the other hand, such a breakdown of self-incompatibility will not be expected in the case of sporophytic pollen control and has not, in fact, been observed (GERSTEL, 1950).

Tetraploid rye is perfectly self-incompatible according to several investigations (*e. g.*, BRESLAWETZ, 1940; LUNDQVIST, 1947; LEWIS, 1954 a).

Following the above-mentioned considerations this has been taken to indicate that sporophytic pollen control occurs in rye (LEWIS, 1954 a). The control of self-incompatibility in rye to be exerted by oppositional alleles is verified cytologically by pollen-tube growth after selfing being strongly inhibited with poor germination of the pollen (SEARS, 1937). A direct genetic verification was obtained recently (LUNDQVIST, 1954); it was suggested, however, that the pollen control might be exerted gametophytically by the operation of two separate incompatibility loci requiring joint identity in the style for both factors of the pollen for full incompatibility reaction to be produced. In mating combinations between siblings in S_1-families obtained from, in all, 15 different diploid population plant clones, the frequency of cross-compatibility was so high — about 60 % — as to warrant the hypothesis of more than one incompatibility locus operating in rye. Upon back-crossing, in the rather few cases tried, the parent plant in question turned out to be completely cross-incompatible as female but highly compatible as male on its offspring. This fact, combined with the high frequency of cross-compatibility, was considered to speak in favour of a gametophytic pollen control (*cf.* BATEMAN, 1952). However, the classification of mating groups and their mutual relationships necessary for a definite decision, was not possible. The experiments have been carried on in 1954 and 1955 in order to obtain more critical information bearing on these questions.

II. MATERIALS AND METHODS

From three of the 15 diploid parental clones previously referred to, all belonging to the commercial variety Steel-rye, *viz.* Nos. 3, 16, and 21 (see LUNDQVIST, 1954, Table 1), offspring groups of various derivations were selected. The selected clones represent different degrees of pseudo-compatibility in the parental material, clone 3 being strongly, clone 16, moderately, and clone 21, rather weakly self-incompatible. 16 and 21 both gave the impression of being heterozygous at both the presumed incompatibility loci; whereas clone 3, the S_1 progeny of which showed a considerably lower frequency of cross-compatibility between siblings, could reasonably be suspected to be heterozygous at only one of these loci.

The material analysed has been summarized in Table 1. Families of the three following different derivations are represented in the table:

TABLE 1. *Material of diploid Steel-rye analysed in 1954 and 1955.*

P = parental clone
S_1- = ♀ incompatible S_1 plant
S_1+ = ♀ compatible S_1 plant
BCr+ = » » plant in progeny derived from $S_1+ \times P$.
S_2+ = » » S_2 plant derived from an S_1+

Parental clone number	Original family number	Year	Family derivation	Parent numbers	Seeds sown	Mature plants tested	Combinations tried	Family number used in text
3	e	1954	$S_1+ \times P$	S_1 3: 3 × P 3	14	14	62	1
	34	1955	BCr+ × BCr+	e: 8 × 1	14	9	30	2
16	a—2	1954	P 16 selfed	P 16 selfed	20	20	65	3
	d—1	»	$S_1+ \times S_1+$	S_1 16: 6 × 5	14	10	39	4
	d—2	»	»	S_1 16: 5 × 6	14	13	38	5
	22	1955	»	a—2: 17 × 9	14	6	27	6
	c—3	1954	$S_1+ \times P$	S_1 16: 5 × P 16	14	9	40	7
	c—4	»	»	S_1 16: 6 × P 16	14	14	50	8
	33	1955	BCr+ × BCr+	c—3: 7 × 4	14	9	20	9
21	a—1	1954	P 21 selfed	P 21 selfed	20	16	48	10
	11	1955	$S_1+ \times S_1+$	a—1: 2 × 16	14	10	36	11
	12	»	»	a—1: 16 × 2	14	12	52	12
	13	»	»	a—1: 16 × 5	14	12	49	13
	14	»	»	a—1: 8 × 12	14	10	44	14
	15	»	$S_1+ \times S_1-$	a—1: 8 × 14	14	6	23	15
	16	»	»	a—1: 12 × 14	14	11	78	16
	c—1	1954	$S_1+ \times P$	S_1 21: 9 × P 21	14	13	36	17
	c—2	»	»	S_1 21: 11 × P 21	14	14	79	18
	27	1955	BCr+ × BCr+	c—1: 13 × 1	14	8	19	19
	32	»	»	c—2: 8 × 1	14	12	60	20
	b—1	1954	S_1+ selfed	S_1 21: 9 selfed	14	12	33	21
	b—2	»	»	S_1 21: 11 selfed	20	17	68	22
	24	1955	S_2+ selfed	b—1: 2 selfed	14	8	24	23

(1) Families derived from a continuous series of selfing, more or less advanced.

(2) Families derived from an S_1 plant, backcrossed to the corresponding parent clone.

(3) Families derived from intercrosses within families of different derivations.

All families were analysed in intrafamily tests; testing of offspring on parent or parents was in no case possible now, as it turned out impracticable after 1953 to save a second-year flowering of the latter. In order to facilitate the orientation in the table, in the last column the original field numbers of the families have been transformed into the consecutive numbering applied in the following.

The poor representation of clone 3 in the table is due to its strong self-incompatibility; thus a second S_1 family could not be raised from this clone. In 1955 the proportion of plants reaching maturity was, in all, considerably lower than in 1954 owing, probably, to more unfavourable sowing conditions; this mortality, however, is not likely to have seriously influenced the representativeness of the material.

The investigation was pursued essentially under the same conditions as previously (see LUNDQVIST, 1954). All crossings were thus made in the greenhouse and included emasculation and manual pollination after control in the microscope of the collected pollen. Only 40—50 of the basal flowers were pollinated; the remaining distal part was removed. The degree of tillering did not permit replications, and thus each crossing combination, as a rule, will comprise 40—50 flowers. For the same reason it was not possible to carry out a complete diallel crossing program.

The classification of mating results was based mainly on the absolute values of seed setting, but allowance was also made for the general vitality of the plant, the development of the ear, and the pollination conditions. The border-line between compatibility and incompatibility has been quite clear, however, as can be seen from Table 2, where the seed setting values from each of the three clone materials are compared. Degrees of self-incompatibility in the parental clone and in its offspring are clearly concordant. In no case were completely self-compatible plants obtained in the offspring families. A slight tendency to overlapping between values classed as compatible and incompatible, respectively (the latter mainly those from selfing), is observable for the material of clone 21 but seems to be of no significance, as the low values

TABLE 2. *Seed setting upon selfing and crossing within the three selected clones and advanced generations. Values classified as incompatible, in bold types.*

×=crossing
⊗=selfing

Parental clone No.	Generation	Per cent seed setting																						
		0	0	5	10	15	20	25	30	35	40	45	50	55	60	65	70	75	80	85	90	95	100
3	⊗ Clone 1952	**73**	**16**																				
	× Offspring 54	**38**	**2**							2	2	1	2	1		4		3	5	1		1	
	⊗ » 54	**12**	**2**																				
	× » 55	**14**		5	2	1	1	1	1			1	2		2								
	⊗ » 55	**9**																					
16	⊗ Clone 1952	**16**	**10**	2																			
	× Offspring 54	**133**	**15+1**	1	4	2	2	2	1	2	1	3	6	7	11	6	7	13	6	8	1		
	⊗ » 54	**49**	**15**	1				1															
	× » 55	**30**	**2**			3		1	2	3		1	1		2	2							
	⊗ » 55	**14**	**1**																				
21	⊗ Clone 1952	**3**	**4**	3	4	2	3		1														
	× Offspring 54	**144**	**25**		1	3	1		5	8	5	4	5	6	11	11	7	12	3	8	5		
	⊗ » 54	**19**	**24**	12	5	5	2	1															
	× » 55	**216**	**64+2**	10+4	1+3	2	1+1	4	4	6	3	5	7	9	11	9	7	9	4		1	1	
	⊗ » 55	**82**	**35**	9	5	1	1																

classed as compatible are mostly derived from small and weak ears, whereas the selfed ear as a rule was the strongest one of the plant.

In order to picture the individual progeny segregations within the families, reciprocally incompatible indivduals have been grouped together, and these primary groups have then been distinguished on the basis of compatibility in any direction or on different reaction with a common tester plant or tester group.

III. NUMBER OF LOCI CONTROLLING SELF-INCOMPATIBILITY

Two S_1 families were analysed in 1954, *viz.* No. 10 from clone 21, and No. 3 from clone 16. The pollination patterns are shown in Fig. 1 *a*.

Family 10. — Six of the plants did not fit unambiguously into the grouping. The remaining 10 plants are divided into 6 groups, *a—f*; it should be observed, however, that the separation of *c* from *f* is not unambigous here but ultimately justified by the continued analysis in an advanced generation. Group *a* fails completely as female but is compatible as male in all combinations tested. All the other groups, at least in some combination, are ♀ compatible. There is a negative, though not significant, correlation between male and female reaction ($r = -0{,}21$; $0{,}5 > P > 0{,}4$). In five cases, individuals from the same intra-incompatible group were tested on a common tester plant or -group; the reactions are concordant. Therefore, data have been concentrated in Fig. 1 *b*, representing each group by a single row and column. Two successive steps of one way inter-compatibility between groups are observed: the ♀ incompatible group *a* is ♂ compatible with *b* and *e*; *b* is ♂ compatible with *e* only; and *e* is the bottom group, compatible as ♀ only. Of the 48 cross combinations, 25 or 52,1 % are compatible.

Family 3. — Six of the plants did not fit unambigously into the grouping, nor is the division of the remaining 14 plants into 6 groups, *a—f*, quite unambigous; group *a* is not based on reciprocal inter-incompatibility, and the separation of *f* from *c* is based upon later obtained evidence. In the combinations tested, group *a* fails as female but is compatible as male. All the other groups, at least in some combination, are ♀ compatible. There is a negative, though not significant, correlation between male reaction and female reaction ($r = -0{,}35$; $0{,}2 > P > 0{,}1$). In 12 cases, individuals from the same intra-incompatible group were tested on a common tester plant or -group, and only once were the reactions ununiform. The exception, plants 2×11, gave no seed setting and may be caused by inadequate pollination conditions. Two successive

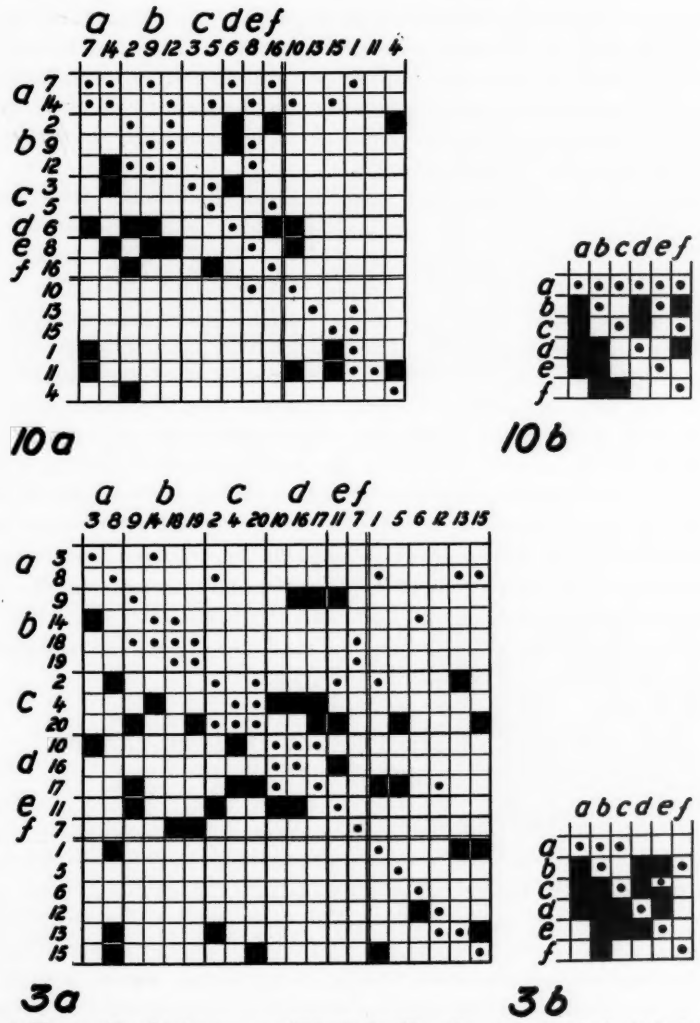


Fig. 1. The results of intercrosses within S_1 families. Rows represent plants tested as females, columns plants tested as males. Plants grouped according to their behaviour. Symbols:

■ compatible pollination.
⊡ incompatible pollination.
Blank: pollination not made.

Actual results to the left (*a*). Interpretation to the right (*b*); each mating group (a—f) being here represented by a single row and column. In both families it was not possible to classify six of the plants, which, accordingly, are not included in the interpretation schemes.

steps of one-way compatibility are observed: the ♀ incompatible group *a* is ♂ compatible with *b* which in its turn gives the same reaction on *f*. The four groups *b*, *c*, *d*, and *e* are inter-compatible in all combinations tried (the combination $b \times c$ is the only one missing). Of 65 cross combinations, 40 or 61,5 % are compatible. Data concentrated, as previously for family 10, are shown in Fig. 1 *b*.

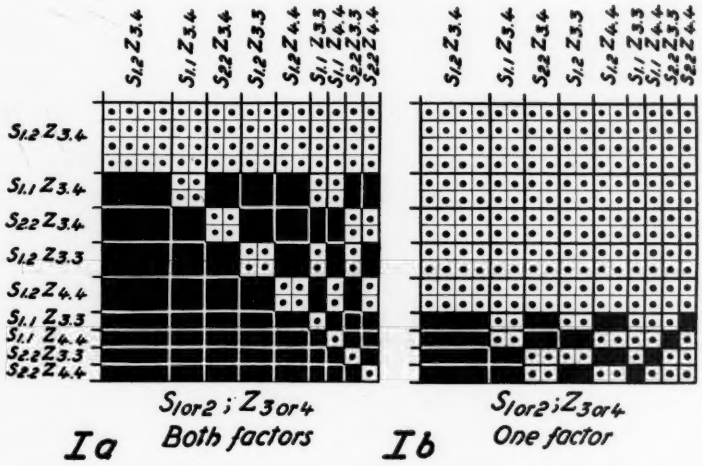
The two S_1-families thus both segregated into more than three groups, intra-incompatible and inter-compatible, indicating the existence of *more than one locus* controlling self-incompatibility. For, in the case of a single incompatibility locus, only three genotypes are possible from selfing of the heterozygote: the heterozygote and the two homozygous genotypes. At two loci after selfing of a dihybrid, 9 different genotypes are possible: the double heterozygote, four different heterozygotes for one locus only, four different double homozygotes. The possibilities to distinguish between these genotypes in a pollination pattern, are dependent on the way of control of the incompatibility reaction in the style and in the pollen, respectively, and on the way of cooperation and interaction between loci, as are also the remaining characteristics of this pattern such as frequency of cross-compatibility, occurrence of one-way compatibility, the uniformity of intra-incompatible groups, and the correlation between the male and the female reactions.

In the following argumentations the existence of two loci controlling self-incompatibility in rye, will be presumed.

IV. THE WAY OF CONTROL OF INCOMPATIBILITY IN THE STYLE

It can be demonstrated (BATEMAN, 1952) that in relation to its S_1 progeny, the parental plant will be ♀ incompatible only following sporophytical control of the incompatibility reaction of the female tissue with independent action of the alleles. (The same applies of course to the heterozygote in such a progeny in relation to its homozygous siblings.) Allelic interaction in the style resulting in dominance or mutual weakening of the incompatibility alleles, will cause ♀ compatibility or self-compatibility of the parent plant. The same effect will also follow from the incompatibility being gametophytically controlled in the style.

On backcrosses (38 cases and 7 parental plants) the parental plant in all cases was ♀ incompatible with its S_1 progeny (LUNDQVIST, 1954). Similarly in the S_1 families analysed above, the groups most successful as males on their siblings are ♀ incompatible. Nor has self-compatibility



Key to the pollen control in model experiments.

I. Gametophytic control at both loci.

II. Sporophytic control at both loci. No dominance interaction.

III. Sporophytic control at both loci. Dominance interaction.

IV. Sporophytic control at the S locus. Gametophytic control at the Z locus.

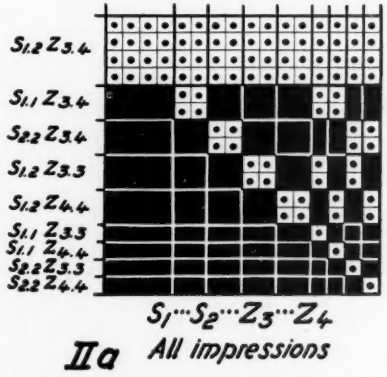


Fig. 2 a. The expected genotypes and their reactions within S_1 families derived from selfing of a plant heterozygous at two incompatibility loci and denoted as $S_{1.2}Z_{3.4}$.

For each scheme the reaction pattern depends on postulated variables, *viz.*:

Upper row: possible factors or »impressions» to be carried by the pollen.

Lower row: factors or impressions of the pollen common to the style, required for a complete incompatibility reaction.

... individual gene action

→ dominance relationship

Other symbols according to Fig. 1.

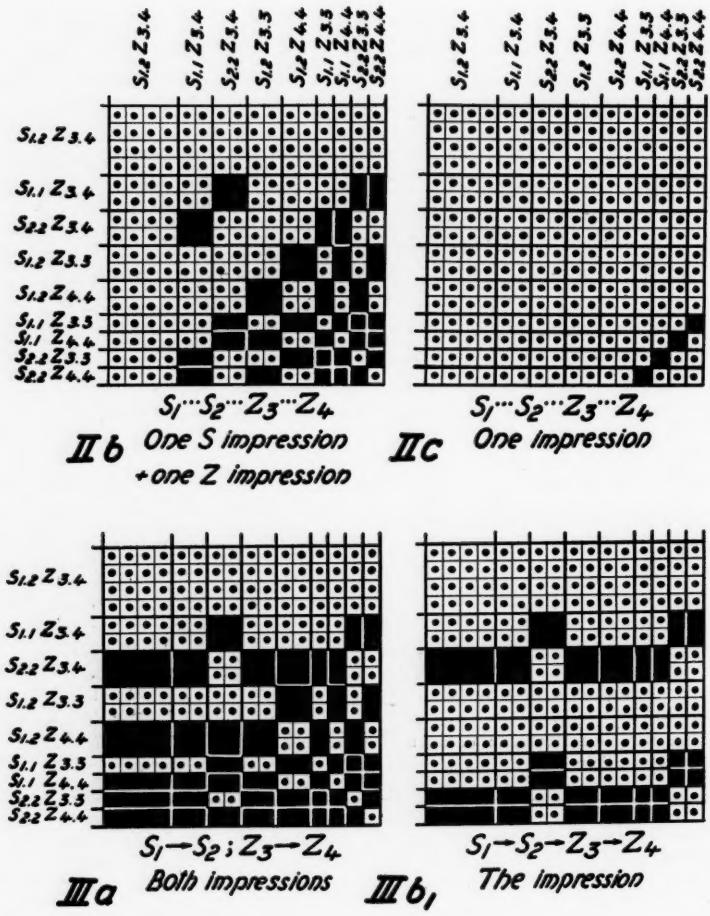


Fig. 2 b. For explanation see legend of Fig. 2 a.

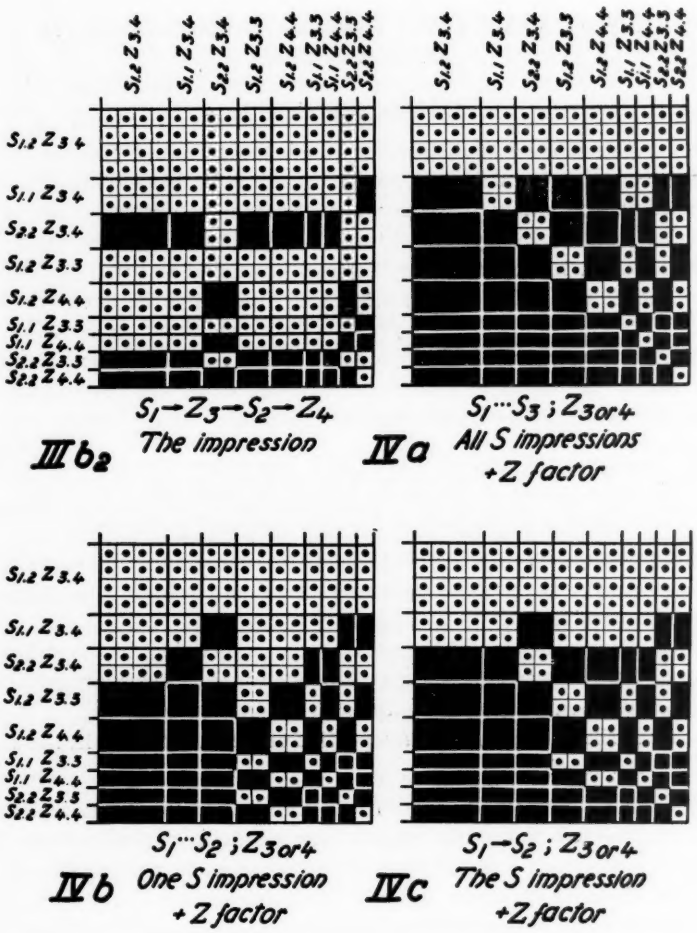


Fig. 2 c. For explanation see legend of Fig. 2 a.

occurred. Thus, data indicate sporophytic incompatibility control in the style with independent action of the alleles.

V. THE WAY OF CONTROL OF INCOMPATIBILITY IN THE POLLEN

1. Model experiments

Four alternative hypotheses for the incompatibility control in the pollen, counting ten different models in all, have been tried. The corresponding pollination patterns within S_1 families derived from selfed parental plants heterozygous at two incompatibility loci, are compared in Fig. 2 *a*—*c*. In the following, the two presumed loci will be denoted as S and Z, respectively, and, arbitrarily, the dihybrid parental plant as $S_{1,2}Z_{3,4}$. The following list presents the conditions required in the different cases for a complete incompatibility reaction to be produced. The list is not complete, but as the extreme cases are covered by the selected models, interpolation may be possible.

(I) Gametophytic control at both loci. The pollen obtains two factors.
  a. Both factors of the pollen have to be matched in the style.
  b. Only one of the factors must be matched in the style.

(II) Sporophytic control at both loci. No dominance interaction between the alleles. The pollen will maximally obtain four different »impressions».
  a. All impressions of the pollen must be matched in the style.
  b. Two impressions of the pollen, one for each locus, are sufficient when matched in the style.
  c. A single impression, irrespective of locus, matched in the style, is sufficient.

(III) Sporophytic control at both loci. Dominance allelic interaction.
  a. The two loci with independent dominance relationships. The pollen will obtain two impressions, requiring joint identity in the style.
  b. A single system of dominance relationships covering both loci. The pollen obtains a single impression.

(IV) The two loci do not react alike. The S locus, *e. g.*, with sporophytic pollen control; the Z locus with gametophytic control. The pollen will maximally obtain two different impressions at S added to the Z factor.

a. The S locus without dominance allelic interaction. In the pollen, all the impressions at S together with the factor at Z, must be matched in the style.

b. The S locus without dominance allelic interaction. In the pollen one of the impressions at S together with the factor at Z must be matched in the style.

c. The S locus with dominance allelic interaction. In the pollen the impression at S together with the factor at Z must be matched in the style.

The question of a possible presence of allelic interaction in the pollen at the gametophytic pollen control, will be considered in a later section. In order to avoid complications, for the present no such interaction will be assumed. As to the alternatives with sporophytic pollen control, we have not considered here the possibility of a mutual weakening of alleles in the heterozygous condition, its effect being self-compatibility or ♀ cross-compatibility of the heterozygote (see BATEMAN, 1952, Table 1).

The characteristics of the ten pollination patterns are summarized in Table 3, as are also the corresponding characteristics of the S_1 families 10 and 3. The frequency of cross-compatibility will increase as the individual locus or allele is incapable of independent production of the incompatibility reaction; the same effect will follow from dominance allelic interaction at the sporophytic control. Only in the case of sporophytic pollen control, when the presence of a single allele common to pollen parent and style parent is sufficient to produce the incompatibility reaction, will it be impossible to distinguish each of the nine possible genotypes. The other characteristics will be evident from a study of the table.

Of the ten alternative models, I *a*, II *a*, and IV *a* are at all essential points of view in a good agreement with the two S_1 families analysed above. The most critical evidence is considered to come from the uniformity of the intra-incompatible groups, observed in both of these families. The case of an intra-incompatible group corresponding to a single genotype, and thus giving rise to a uniform incompatibility reaction pattern, is represented only by I *a*, II *a*, and IV *a*.

Thus, under the assumption of two incompatibility loci, data so far obtained may be interpreted in three different ways:

TABLE 3. *Characteristics of incompatibility reactions within S_1 families derived from selfing of a plant heterozygous at two incompatibility loci. Oppositional incompatibility systems according to Ia—IVc in text, assumed. Sporophytic control with independent gene action postulated for the style. S and Z denote the two loci; G and Sp, gametophytic and sporophytic control; I and D, independent gene action and dominance interaction. Identities between style and pollen factors or »impressions» required for a full incompatibility reaction, indicated. Actual data from S_1 families 10 and 3, compared.*

Locus:	S Z	S Z	S Z	S Z	S Z	S Z	S Z	S Z	S Z	S Z		
Control:	G G	G G	Sp Sp	Sp Sp	Sp Sp	Sp Sp	Sp Sp	Sp G	Sp G	Sp G		
Interaction:	—	—	I	I	I	D+D	D	I —	I —	D —		
Identity in style required:	1+1	1	all+all	1+1	1	1+1	1	all+1	1+1	1+1		
Family number:	Ia	Ib	IIa	IIb	IIc	IIIa	IIIb 1 or 2	IVa	IVb	IVc	10	3
Number of mating groups possible	9	9	9	9	5	9	3 or 6	9	9	9	6	6
% compatible combinations	60,9	14,1	60,9	23,5	1,6	43,8	25,0	60,9	45,3	53,1	52,1	61,5
One-way compatibility	+	+	+	—	—	+	+	+	+	+	+	+
More than one genotype in an intra-incompatible group	—	+	—	+	+	+	+	—	+	+	—	(—)
Correlation between ♂ and ♀ reaction	—	—	—	+	+	No	No	—	—	—	— 0,21	— 0,35

(1) Pollen control is gametophytic. The two alleles of the pollen, one S allele, and one Z allele must both be matched in the style to produce incompatibility.

(2) Pollen control is sporophytic with independent action of the alleles. Maximally four different impressions of a pollen grain are possible, two from the S locus and two from the Z locus. Identity is required between style and all the impressions carried by a particular pollen grain for incompatibility to be produced.

(3) At one locus pollen control is sporophytic with independent action of the alleles, at the other locus, it is gametophytic. The pollen obtains maximally two different sporophytic impressions added to one gametophytic factor. Complete identity with the style is required to produce incompatibility.

These three alternatives will show identical pollination patterns. The frequency of cross-compatibility will be 60,9 %. The correlation between ♂- and ♀-reaction is clearly negative; the more heterozygous the plant at the incompatibility loci, the more frequently will it be successful as male and the less frequently, effective as female to its siblings. The two successive steps of one-way inter-compatibility observed within the two S_1 families will thus be explained; they correspond to the three levels of heterozygosity possible in the progeny of a selfed dihybrid: the level of double heterozygosity, of single heterozygosity and of double homozygosity, the two latter each with 4 different genotypes possible. The double heterozygote $S_{1.3}Z_{3.4}$ is equivalent to the parent plant and thus completely ♀ incompatible, but ♂ compatible with all the other genotypes. Those heterozygous for one locus only, are ♂ compatible with all the other genotypes except $S_{1.3}Z_{3.4}$. The double homozygous genotypes are ♂ incompatible with $S_{1.3}Z_{3.4}$ and also with some genotypes from the singly heterozygous category.

The progeny from different combinations within and between these levels may be analysed now. Following the remaining alternative hypotheses for the incompatibility control in the pollen, reciprocally inter-incompatible individuals must belong to the same genotype, and thus the number of incompatibility groups possible will always be equal to the number of genotypes possible. This is of essential importance for the analysis, for so it will be possible in advanced generations to pursue the discrimination between gametophytic and sporophytic pollen control that could not be settled definitely from a study of the S_1 family itself.

The following comparison between the three possible alternatives will

♀ ♂ ♀ ♂

1. ho ho×ho ho (and recipr.)

$$\begin{array}{lll} A & & \\ A & S_{1.1}Z_{3.3}\times & \left\{\begin{array}{lll} S_{1.1}Z_{4.4}\rightarrow & S_{1.1}Z_{3.4} & \leftarrow S_{1.1}Z_{4.4} \\ S_{2.2}Z_{3.3}\rightarrow & S_{1.2}Z_{3.3} & \leftarrow S_{2.2}Z_{3.3} \\ S_{2.2}Z_{4.4}\rightarrow & S_{1.2}Z_{3.4} & \leftarrow S_{2.2}Z_{4.4} \end{array}\right\}\times S_{1.1}Z_{3.3} \\ A & & \end{array}$$

2. ho ho×ho het (and recipr.)

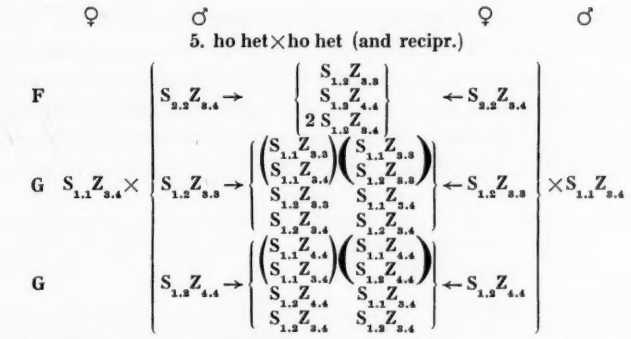
$$\begin{array}{lll} B & & \left\{\begin{array}{lll} S_{1.1}Z_{3.4}\rightarrow \left\{\begin{array}{l}(S_{1.1}Z_{3.3}) \\ S_{1.1}Z_{3.4}\end{array}\right. & \text{——} & \leftarrow S_{1.1}Z_{3.4} \\ S_{2.2}Z_{3.4}\rightarrow & \left\{\begin{array}{l}S_{1.2}Z_{3.3} \\ S_{1.2}Z_{3.4}\end{array}\right. & \leftarrow S_{2.2}Z_{3.4} \\ S_{1.2}Z_{3.3}\rightarrow \left\{\begin{array}{l}(S_{1.1}Z_{3.3}) \\ S_{1.2}Z_{3.3}\end{array}\right. & \text{——} & \leftarrow S_{1.2}Z_{3.3} \\ S_{1.2}Z_{4.4}\rightarrow & \left\{\begin{array}{l}S_{1.1}Z_{3.4} \\ S_{1.2}Z_{3.4}\end{array}\right. & \leftarrow S_{1.2}Z_{4.4} \end{array}\right\}\times S_{1.1}Z_{3.3} \\ C & S_{1.1}Z_{3.3}\times & \\ B & & \\ C & & \end{array}$$

3. ho ho×het het (and recipr.)

$$D\quad S_{1.1}Z_{3.3}\times S_{1.2}Z_{3.4}\rightarrow \left\{\begin{array}{l}(S_{1.1}Z_{3.3}) \\ S_{1.1}Z_{3.4} \\ S_{1.2}Z_{3.3} \\ S_{1.2}Z_{3.4}\end{array}\right. \text{——} \leftarrow S_{1.2}Z_{3.4}\times S_{1.1}Z_{3.3}$$

4. ho het×het het (and recipr.)

$$\begin{array}{lll} E & & \left\{\begin{array}{lll} S_{1.1}Z_{3.4}\rightarrow \left\{\begin{array}{l}\left(\begin{array}{l}S_{1.1}Z_{3.3} \\ S_{1.1}Z_{4.4}\end{array}\right) \\ 2\,S_{1.1}Z_{3.4} \\ S_{1.2}Z_{3.3} \\ S_{1.2}Z_{4.4} \\ 2\,S_{1.2}Z_{3.4}\end{array}\right. & \text{——} & \leftarrow S_{1.1}Z_{3.4} \\ S_{2.2}Z_{3.4}\rightarrow \left\{\begin{array}{l}\left(\begin{array}{l}S_{2.2}Z_{4.4} \\ S_{2.2}Z_{3.3}\end{array}\right) \\ 2\,S_{2.2}Z_{3.4} \\ S_{1.2}Z_{3.3} \\ S_{1.2}Z_{4.4} \\ 2\,S_{1.2}Z_{3.4}\end{array}\right. & \text{——} & \leftarrow S_{2.2}Z_{3.4} \\ S_{1.2}Z_{3.3}\rightarrow \left\{\begin{array}{l}\left(\begin{array}{l}S_{1.1}Z_{3.3} \\ S_{2.2}Z_{3.3}\end{array}\right) \\ 2\,S_{1.2}Z_{3.3} \\ S_{1.1}Z_{3.4} \\ S_{2.2}Z_{3.4} \\ 2\,S_{1.2}Z_{3.4}\end{array}\right. & \text{——} & \leftarrow S_{1.2}Z_{3.3} \\ S_{1.2}Z_{4.4}\rightarrow \left\{\begin{array}{l}\left(\begin{array}{l}S_{1.1}Z_{4.4} \\ S_{2.2}Z_{4.4}\end{array}\right) \\ 2\,S_{1.2}Z_{4.4} \\ S_{1.1}Z_{3.4} \\ S_{2.2}Z_{3.4} \\ 2\,S_{1.2}Z_{3.4}\end{array}\right. & \text{——} & \leftarrow S_{1.2}Z_{4.4} \end{array}\right\}\times S_{1.2}Z_{3.4} \\ E & & \\ E & S_{1.2}Z_{3.4}\times & \\ E & & \\ E & & \end{array}$$



Scheme 1. Different sib mating categories possible within an S_1 family derived from selfing of a plant heterozygous at two incompatibility loci, and the resultant genotypes in the offspring. Incompatibility systems according to I a, II a, and IV a in text, assumed. Inhibition of part of the pollen, possible on the schemes of I a and IV a. Genotypes within brackets, possible only on the schemes of II a and IV a; those within bold type brackets, only on the purely sporophytic scheme of pollen control, II a. (A—G refer to the mating categories of Table 4. For genotype symbols, see text.)

first consider the two hypotheses that imply purely gametophytic and purely sporophytic pollen control, respectively.

2. Purely gametophytic or purely sporophytic pollen control?

On the gametophytic scheme of pollen control, the pollen will react quite according to its own genotype, and thus in a particular combination, part of the pollen may function, whereas the other part may be inhibited by an incompatibility reaction. In such case, the segregation will be incomplete, some of the genotypes not being obtained in the offspring. On the sporophytic scheme of pollen control, on the other hand, the sporophyte will impress all its pollen with the same phenotype; if a particular cross combination is compatible at all, all pollen will react alike and function, and no genotypes will be missing in the progeny.

The nine possible genotypes in an S_1 family derived from a plant of the genotype $S_{1.2}Z_{3.4}$, correspond to the double heterozygote, four single heterozygotes, and four double homozygotes. Five different combinations are possible within and between these three levels of heterozygosity; the corresponding resultant progeny genotypes, expected on the gametophytic and the sporophytic scheme, respectively, are illustrated with completely executed examples in Scheme 1. It would of course be desirable to denote the plants tested in the particular combinations with

TABLE 4. *Different sib mating categories possible within an S_1 family derived from selfing of a plant heterozygous at two incompatibility loci, and the resultant number of genotypes in the offspring. Incompatibility systems according to I a and II a in text, assumed. Inhibition of part of of the pollen, possible only on the gametophytic scheme of pollen control. (A—G refer to the mating categories of Scheme 1. For genotype symbols, see text.)*

Mating combination	Number of gametic genotypes		Gametophytic pollen control		Sporophytic pollen control
	Female side	Male side	Pollen genotypes uninhibited	Genotypes in the offspring	
A.					
ho ho × ho ho	1	1	1	1	1
(recipr.)	1	1	1	1	1
B.					
ho_aho × ho_ahet	1	2	1	1	2
ho_ahet × ho_aho	2	1	—	—	—
C.					
ho ho × ho het	1	2	2	2	2
ho het × ho ho	2	1	1	2	2
D.					
ho ho × het het	1	4	3	3	4
het het × ho ho	4	1	—	—	—
E.					
ho het × het het	2	4	2	3	6
het het × ho het	4	2	—	—	—
F.					
ho_ahet × ho_bhet	2	2	2	3	3
ho_bhet × ho_ahet	2	2	2	3	3
G.					
ho het × het ho	2	2	1	2	4
het ho × ho het	2	2	1	2	4

adequate genotype formulas combined from the four alleles of the original parent plant. As will be seen below, data are somewhat fragmentary, seldom permitting more than a safe determination of the level of heterozygosity applicable to the plants in question, and only in some few cases lining out more exact genotypic relationships within a limited

TABLE 5. *Frequencies expected on different schemes of pollen control (systems I a and II a, in text) for different numbers of genotypes to segregate from reciprocally compatible S_1 sibs of unknown constitution, derived from a plant heterozygous at two incompatibility loci.*

Relative frequency at	Numbers of genotypes			
	1	2	3	4
Gametophytic pollen control	6/46	32/46	8/46	—
Sporophytic » »	6/46	16/46	8/46	16/46

number of plants. Therefore it has been considered convenient as a substitute to introduce the following denotations:

ho ho; homozygosity at both loci
ho het; homozygosity at one of the loci
het het; heterozygosity at both loci.

These denotations constitute a general expression of the level of heterozygosity. Suffices or marking off of the locations will add to a more detailed description when possible or necessary:

ho_ahet and ho_bhet, respectively; homozygosity at one of the loci for one or the other, respectively, of the two alleles.
$\underline{ho}$ het and het $\underline{ho}$, respectively; homozygosity at the first or the second, respectively, of the two loci.

Scheme 1 is summarized in Table 4. In most cases the sporophytic pollen control is accompanied by a considerably higher number of genotypes in the progeny, always at least equalling the number possible on the gametophytic scheme. As can be seen from the table, one-way inter-compatibility indicates different levels of heterozygosity. On the other hand, reciprocal inter-compatibility is not limited to plants from the same level but is possible between different levels also. The number of segregating genotypes possible in the progeny derived from reciprocally compatible combinations will vary with different combinations; the different frequencies of the numbers of genotypes expected on the two schemes of pollen control are compared in Table 5.

A. Analysis of families derived from S_1 plants

Available are families derived from intercrosses between S_1 siblings or from backcrosses of S_1 plants to the respective parent clones.

Intercross families. — In all, nine families are analysed; six (Nos. 11—

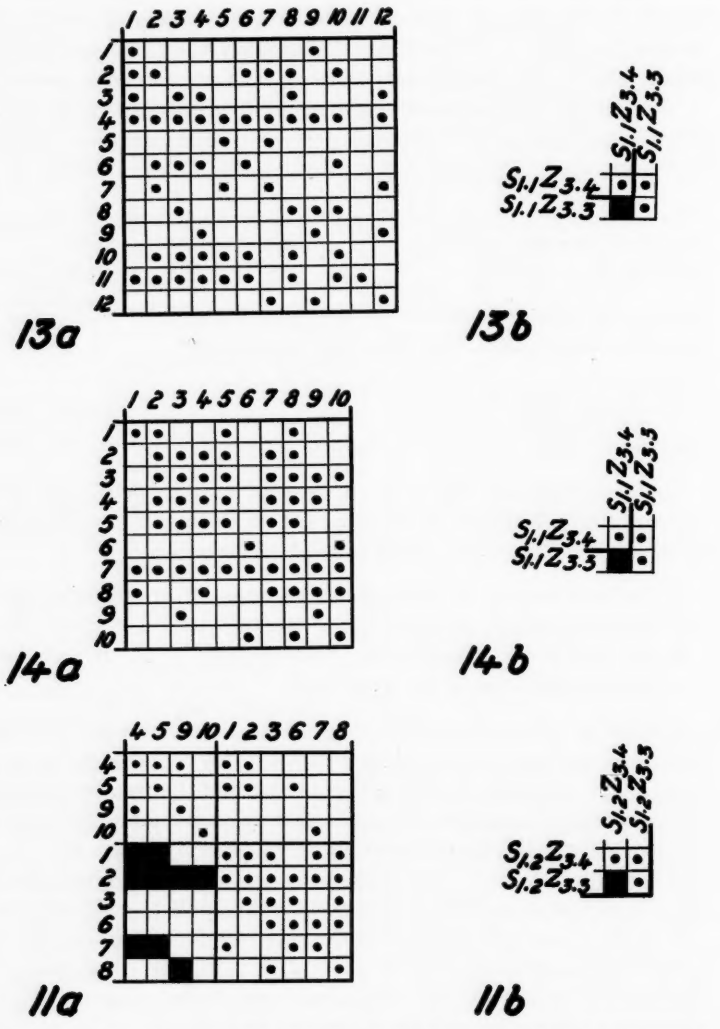


Fig. 3. Results of intercrosses within families derived from S_1 siblings intercrossed. Rows represent plants tested as females, columns, plants tested as males. Plants grouped according to their behaviour. Symbols according to Fig. 1.

Actual results to the left (a). Expected genotypes and their reactions to the right (b); each genotype is here represented by a single row and column. Expected on the gametophytic scheme, upper left corner bounded by bold lines. Expected on the sporophytic scheme, the whole pattern.

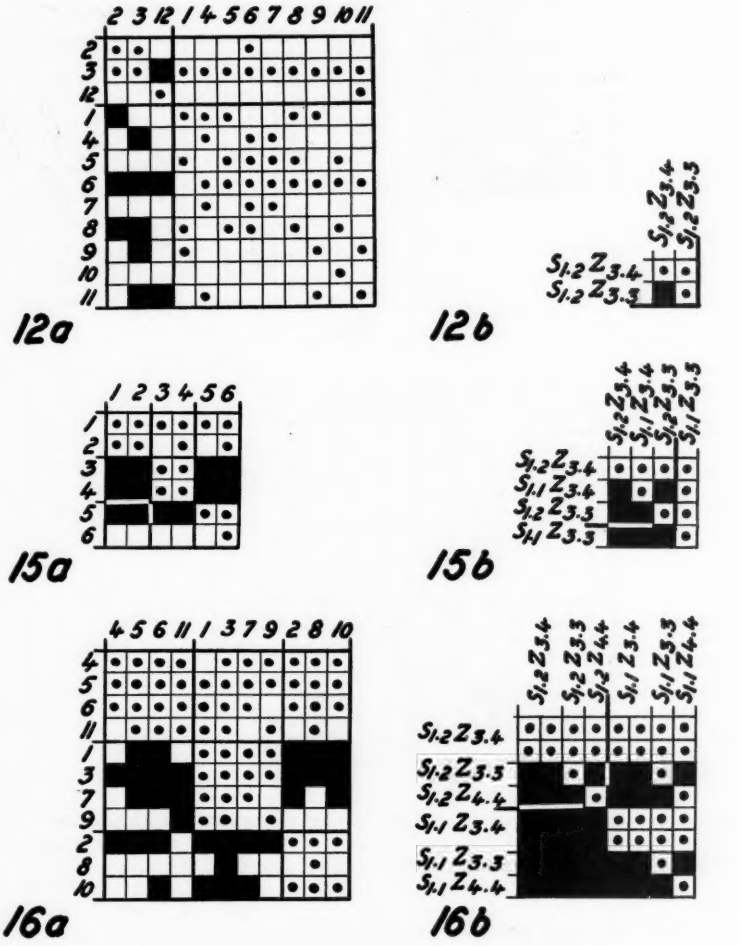


Fig. 4. Results from intercrosses within families derived from S_1 siblings intercrossed. Rows represent plants tested as females, columns, plants tested as males. Plants grouped according to their behaviour. Symbols according to Fig. 1.

Actual results to the left (a). Expected genotypes and their reactions to the right. Expected on the gametophytic scheme, upper left corner, bounded by bold lines Expected on the sporophytic scheme, the whole pattern.

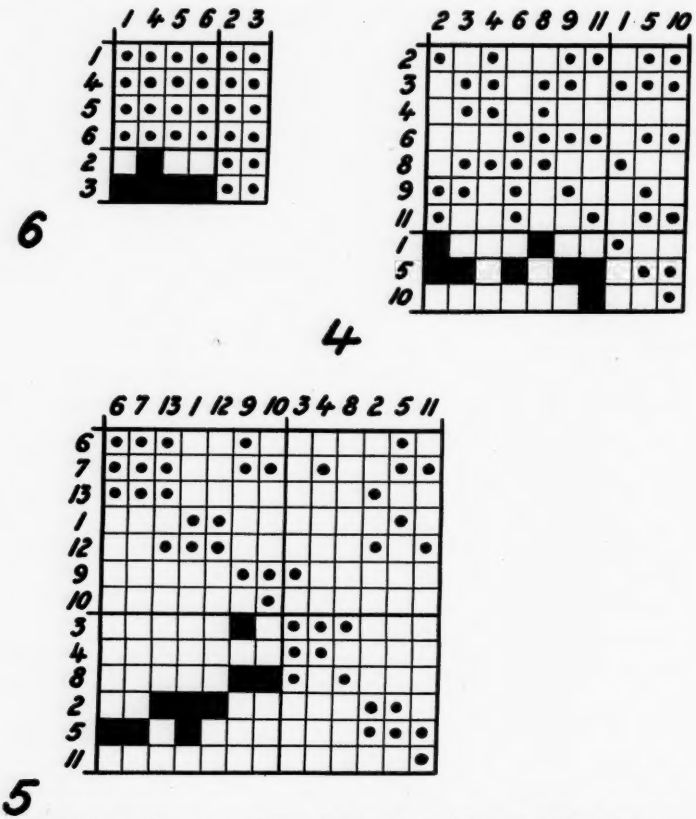


Fig. 5. Results from intercrosses within families derived from S_1 siblings intercrossed. Rows represent plants tested as females, columns, plants tested as males. Plants grouped according to their behaviour. Symbols according to Fig. 1.

16) derived from combinations within the S_1 family 10; one (No. 6) from the S_1 family 3; and two (Nos. 4 and 5) from the primary S_1 family of clone 16; grown in 1953. The nine corresponding pollination patterns are compared in Figs. 3—5, those of families 11—16 together with the pollination patterns theoretically expected on the gametophytic or the sporophytic scheme of pollen control. In the latter patterns the indicated genotypes do not imply specified relationships between the particular families but only within the individual family. The experimental data are summarized in Table 6 *a*.

TABLE 6. *Origin and constitution of families derived from S_1 plants intercrossed or backcrossed to parent clone. Numbers of genotypes observed compared with those expected on the two schemes of pollen control assumed (systems I a and II a, in text).*

Category of Table 4	Pollination pattern	Clone number	Family number	Parent genotypes	Number of groups in progeny			Numbers of plants	
					Expected when pollen control is		Observed		
					gametoph.	sporoph.		♀ incomp.	♀ comp.
a. Intercross S_1 sib × S_1 sib									
B	Fig. 3	21	13	$ho_a ho \times ho_a het$	1	2	1	12	—
»	»	»	14	» »	1	2	1	10	—
C	»	»	11	$ho_b het \times ho_a ho$	2	2	2	4	6
»	Fig. 4	»	12	$ho_a ho \times ho_b het$	2	2	2	3	9
D	»	»	15	ho ho × het het	3	4	3	2	2+2
E	»	»	16	ho het × het het	3	6	3	4	4+3
F or G	Fig. 5	16	6	ho het × ho het	3 or 2	3 or 4	2	4	2
A, C, F, or G	»	»	4	ho ho × ho ho $ho_a ho \times ho_b het$	1, 2, or 3	1, 2, 3, or 4	2	7	3
» » » »	»	»	5	ho het × ho het	1, 2, or 3	1, 2, 3, or 4	2	7	6
b. Backcross S_1 × Parent clone									
D or E	Fig. 6	21	17	ho ho or ho het × het het	3	4 or 6	3	4	4+3
»	»	»	18	» »	3	4 or 6	3	6	5+3
»	»	16	7	» »	3	4 or 6	3	6	2+1
»	»	»	8	» »	3	4 or 6	2	8	6
B, D, or E	»	3	1	$ho_a ho \times ho_a het$ ho ho or ho het × het het	1 or 3	2, 4, or 6	3	9	2+3

Within the S_1 family 10 the genotypic relationships may be evolved as follows (see Fig. 1). Group *a* being completely ♀ incompatible but ♂ compatible with all other groups tested to it, is het het. All the other groups must be homozygous at least at one locus. Group *b* is the constituent of two one-way inter-compatible combinations, being ♀ compatible to *a* but ♂ compatible on *e*. Thus it seems to have a number of different incompatibility alleles lower than that of group *a*, but higher than that of *e* and must be considered as ho het, group *e* being ho ho. Plant 5 must carry a number of different alleles higher than that of plant 16, being one-way compatible, as male on the latter, and cannot thus be ho ho. Moreover, it is ♂ incompatible on plant 3, that cannot be het het being ♀ compatible in some combinations. Plant 16 being ho ho, plants 5 and 3 must thus be ho het, belonging to the same genotype (group *c*). The genotype of plant 6 is left undetermined.

Summing up:

het het	—	7 and 14
ho het	—	2, 9, and 12; 3 and 5
ho ho	—	8; 16
undetermined	—	6

Four of the families analysed are derived from combinations of the category ho ho×ho het. Plant 16, considered as ho ho, is reciprocally compatible with plant 2, and the two resultant families (11 and 12) thus represent category *C* of Table 4 and scheme 1. One-way compatibility results when plant 16 is combined with plant 5, the latter being ho het; the resultant family 13 thus represents category *B*. The same relations obtain for plant 8, ho ho, combined with plant 12, and their resultant family 14. The plants 8, 12, and 14 constitute a system of two successive steps of one-way inter-compatibility. Of the following combinations, 8×12 (family 15) represents category *D*, and 12×14 (family 16), category *E*, plant 14 being considered as het het.

Within the S_1 family 3 the relationships are less accurately determinable (see Fig. 1). All groups except *a*, are ♀ compatible at least in some combinations. The groups *b*, *c*, *d*, and *e* are diallely inter-compatible (11 of the 12 possible combinations tried). Scheme 1 will show that such a system of 4 inter-compatible groups is only possible when all plants concerned belong to the same level of heterozygosity, either ho ho or ho het. Within each of these levels four different genotypes are possible. Group *b* is one-way compatible as male on group *f* and is therefore considered to be ho het, whereas group *f* must be ho ho. Group *a* is ♀ in-

compatible; being ♂ compatible on the ho het groups *b*—*d*, it must be het het.

Summing up:

het het	—	3 and 8
ho het	—	9, 14, 18, and 19; 2, 4, and 20; 10, 16, and 17; 11
ho ho	—	7

Materials from group *b* (plant 9) combined as male with the three remaining groups of ho het were grown for an analytical test of point 5 of Scheme 1. Of the three families, however, after various mishaps, two became so reduced as to make them useless; the remaining one is family 6.

Within the primary S_1 families of 1953 the possibilities of classifying genotypes were very limited; after sibling intercrosses and backcrosses to the parent plant, the plants could be grouped into two categories: ♀ compatible, and ♀ incompatible. Those compatible as female must be homozygous, at least at one of the incompatibility loci. The analysed families 4 and 5 are the reciprocals from a sib combination compatible in both directions; both siblings were thus homozygous at least at one locus.

Backcross families. — Backcrosses of S_1 plants to parent clone were possible only in 1953. All three clones are represented here, including, in all, five families, Nos. 1, 7, 8, 17, and 18. The five corresponding pollination patterns are compared in Fig. 6. The experimental data are summarized in Table 6 *b*.

Of the three parental clones, P 16 and P 21 were already shown to be heterozygous at both incompatibility loci. Backcrosses of S_1 plants to these parent clones will, therefore, represent category *D* or *E* in Table 4 and Scheme 1. Clone P 3, on the other hand, may be suspected to be heterozygous at only one of the loci, judging from the relatively low frequency of compatible combinations between S_1 siblings and in backcrosses of S_1 plants to parent plant. In such a case, the compatible backcrosses being equal to $ho_a ho \times ho_a het$ (category *B* in table 4) would give rise to a single genotype in the progeny.

Conclusions. — On the whole the results are quite clear cut. Some few cases are somewhat ambiguous. In family 12, plant 3 gave some seed setting as female to plant 12, incompatibility being expected here. However, the seed setting is relatively low — 22,5 % — and may possibly be explained by the two plants concerned being the most pseudo-fertile ones of family 12. In family 17, plants 8 and 9 are not definitely

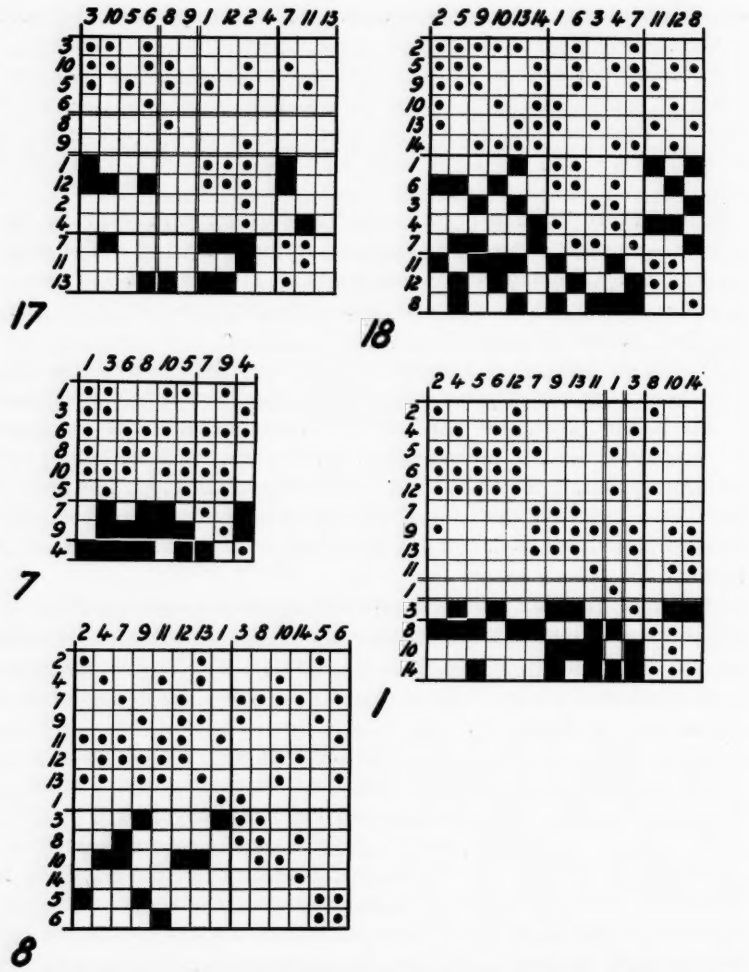


Fig. 6. Results from intercrosses within families derived from S_1 siblings back-crossed to parent clone. Rows represent plants tested as females, columns, plants tested as males. Plants grouped according to their behaviour. Symbols according to Fig. 1.

Unclassified are in family 1 plant 1, and in family 17, plants 8 and 9. They are, therefore, separated from the two groups to which they might belong, by double lines. In family 17 plants 9, 4, and 13 were male sterile and could not be tested as males.

classified, and in the pollination pattern they are thus placed between the two groups to which they might belong. In family 8, plant 5 by plant 2 gave poor seed setting, 4,2 %, compatibility being expected here. However, this pollinated ear was the weakest one of plant 5, and as the selfed ear was completely sterile it may be justifiable to classify the combination as compatible. In family 1, plant 1 is not definitely classified, therefore being placed between the two groups possible.

Otherwise the pollination patterns are quite regular, reciprocally inter-incompatible plants reacting uniformly. In all families but one (family 8), the reaction patterns agree completely with those expected on two incompatibility loci with gametophytic pollen control and the combined parental S_1 plants belonging to the genotypes presumed. This applies to the number of segregating groups and to their individual incompatibility relationships also. Nor do the frequencies of the different groups differ significantly from those expected for the particular cases in Scheme 1. It must, however, be pointed out that the latter conclusion is arrived at only by inference as it has not been possible to check directly the underlying genotypes, owing to the difficulty to conserve tester genotypes from year to year. In no case are data more adequately interpreted on the scheme of sporophytic pollen control. In the deviating family 8 only two groups can be distinguished, but within the ♀ compatible group of this family some critical mating combinations are lacking, and thus even this family may perfectly well in fact have segregated into the three groups expected.

Clone 3, also, must have been a heterozygote at both loci, as three groups are obtained in the progeny (family 1) from an S_1-plant backcrossed as female to P 3. If P 3 were heterozygous at only one of the loci, the combination above would correspond to category *B* of Table 4, thus giving rise to only one group in the offspring.

In Table 6 *a*, there remain three families (6, 4 and 5) derived from the combination of reciprocally compatible S_1 siblings, where the exact number of groups expected could not be settled in advance. Two groups were obtained, this being the most frequent number in the offspring from reciprocally compatible S_1 siblings combined, when pollen control is gametophytic (see Table 5).

It may be objected here that a discrepancy between data observed, and expected on the hypothesis of sporophytic pollen control, might arise from the number of plants tested being sometimes rather limited; in other cases the incomplete combination schemes with their frequent gaps might lack some critical combinations necessary for a critical test

of the segregation. Thus, owing to mere chance, some of the genotypes possible might not have been obtained at all or might not be distinguished in the families analysed. The true existence of a sporophytic pollen control might also be concealed by elimination of certain genotypes at the zygotic stage, giving a delusive impression of a gametophytic pollen control operating. It will thus be necessary in the families analysed above to demonstrate beyond doubt the absence of genotypes possible only on the sporophytic scheme of pollen control. Regarding this question the backcross families of Table 6 *b* were further tested.

B. Analysis of families derived from reciprocally compatible intercrosses within backcross families

Starting from four of the five backcross families previously studied, the three clones will all be represented in this analysis. The clones have all been demonstrated to be heterozygous at both loci, a compatible backcross of an S_1 individual thus representing the categories 3 or 4 of Scheme 1, *i. e.*, ho ho × het het, and ho het × het het, respectively. As far as could be determined, each of the four backcross families segregated into three groups. Of these, one was completely ♀ incompatible but ♂ compatible on the other two, the latter in addition being reciprocally compatible. For the argumentation to be followed more easily, the categories 3 and 4 from Scheme 1 are reproduced below:

3. ho ho × het het

$S_{1.1}Z_{3.3} \times S_{1.2}Z_{3.4}$

$(S_{1.1}Z_{3.3})$	(Possible only when pollen control is sporophytic)
$S_{1.1}Z_{3.4}$	
$S_{1.2}Z_{3.3}$	
$S_{1.2}Z_{3.4}$	

4. ho het × het het

$S_{1.1}Z_{3.4} \times S_{1.2}Z_{3.4}$

$\begin{pmatrix} S_{1.1}Z_{3.3} \\ S_{1.1}Z_{4.4} \\ 2\ S_{1.1}Z_{3.4} \end{pmatrix}$	(Possible only when pollen control is sporophytic)
$S_{1.2}Z_{3.3}$	
$S_{1.2}Z_{4.4}$	
$2\ S_{1.2}Z_{3.4}$	

Assuming sporophytic pollen control and an offspring family from the backcross combination ho ho × het het that segregates into three groups, it can be seen from the scheme above that the only way of obtaining the reciprocal inter-compatibility observed, is the combination of the two genotypes heterozygous at one locus. The third group being

Backcross family	Progeny from reciprocally compatible groups of backcross family	
Constitution	Number of groups possible	Genotypes possible
1. $S_{1.1}Z_{3.3}$, $S_{1.1}Z_{4.4}$, $S_{1.1}Z_{3.4}$ →	1	$S_{1.1}Z_{3.4}$
2. $S_{1.1}Z_{3.3}$, $S_{1.1}Z_{4.4}$, $S_{1.2}Z_{3.4}$ →	1	$S_{1.1}Z_{3.4}$
3. $S_{1.1}Z_{4.4}$, $S_{1.2}Z_{3.3}$, $S_{1.2}Z_{3.4}$ →	2	$S_{1.1}Z_{3.4}$ $S_{1.2}Z_{3.4}$
4. $S_{1.1}Z_{3.3}$, $S_{1.2}Z_{4.4}$, $S_{1.2}Z_{3.4}$ →	2	$S_{1.1}Z_{3.4}$ $S_{1.2}Z_{3.4}$
5. $S_{1.2}Z_{3.3}$, $S_{1.2}Z_{4.4}$, $S_{1.2}Z_{3.4}$ →	3	$S_{1.1}Z_{3.4}$ $S_{2.2}Z_{3.4}$ $2\,S_{1.2}Z_{3.4}$
6. $S_{1.1}Z_{3.4}$, $S_{1.2}Z_{4.4}$, $S_{1.2}Z_{3.4}$ →	4	$S_{1.1}Z_{4.4}$ $S_{1.2}Z_{4.4}$ $S_{1.1}Z_{3.4}$ $S_{1.2}Z_{3.4}$
7. $S_{1.1}Z_{3.4}$, $S_{1.2}Z_{3.3}$, $S_{1.2}Z_{3.4}$ →	4	$S_{1.1}Z_{3.3}$ $S_{1.2}Z_{3.3}$ $S_{1.1}Z_{3.4}$ $S_{1.2}Z_{3.4}$

Scheme 2. Origin and constitution of progeny from reciprocally compatible plants in backcross families of varying constitution. Constitution based on the six genotypes possible on the sporophytic scheme of pollen control (system II *a*, in text) when the parent plant is het het $S_{1.2}Z_{3.4}$ and the backcrossed S_1 plant is ho het $S_{1.1}Z_{3.4}$. For further explanation see text.

compatible only as male must then be het het. If, among the four backcross families studied category 3 above be represented, an elimination of the double homozygous genotype must in some way be presumed. When the backcross belongs to the type ho het × het het, there are seven different ways possible to eliminate in the offspring three genotypes at a

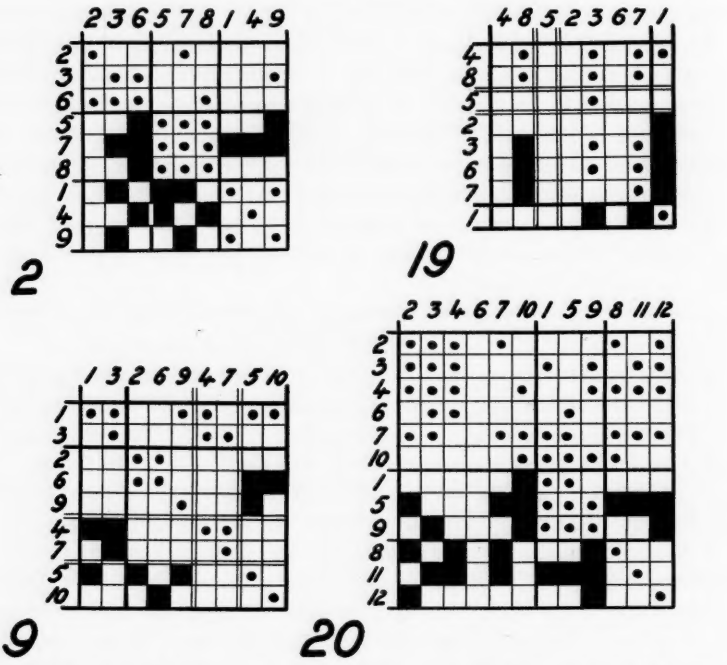


Fig. 7. Results from intercrosses within families derived from reciprocally compatible siblings in backcross families of Fig. 6. Rows represent plants tested as females, columns, plants tested as males. Plants grouped according to their behaviour. Symbols according to Fig. 1.

Unclassified are in family 9, plants 4 and 7, and in family 19, plant 5. They are, therefore, separated from the two groups to which they might belong, by double lines. In family 19, plants 2, 4, 5, and 6 and in family 20, plant 6, were male sterile and could not be tested as males.

time from the six possible genotypes in order to obtain the actual pollination pattern within the backcross family with one group ♀ incompatible but ♂ compatible, and the other two groups reciprocally inter-compatible. These seven alternatives are compared in Scheme 2. In the alternatives 1—4, at least one of the reciprocally compatible groups in the backcross family is double homozygous. The alternatives 6 and 7 are identical with the composition demonstrated above for the offspring from the backcross ho ho×het het.

The pollination patterns of the four progeny families studied (Nos. 2, 9, 19, and 20), derived from reciprocally compatible plants within each

TABLE 7. *Origin and constitution of families derived from reciprocally compatible siblings (BCr+ × BCr+) within backcross families (S₁+ × P).*

Clone number	Family number	Parent numbers	Number of groups	Number of plants	
				♀ incompatible	♀ compatible
21	19	c—1 : 13 × 1	3	2	1 + 4
»	20	c—2 : 8 × 1	3	6	3 + 3
16	9	c—3 : 7 × 4	3	2	3 + 2
3	2	e—8 : 8 × 1	3	3	3 + 3

of the four backcross families above, are compared in Fig. 7, and data are summarized in Table 7. They do not permit an unambiguous interpretation owing to the numerous gaps in the pollination schemes. However, the following facts are essential for the interpretation of data.

(1) Cases of one-way compatibility occur in all families. An individual which is ♂ compatible in a one-way compatible combination, is in no case ♀ compatible.

(2) Cases of reciprocal compatibility occur in all families.

(3) At least three groups are thus obtained in all families; but in no case will it be necessary to assume more than these three groups.

The occurrence of more than one genotype within each of the four families will eliminate the alternatives 1 and 2 of Scheme 2; cases of reciprocal compatibility will exclude the alternatives 3 and 4; the occurrence of only two levels of heterozygosity can result from the alternatives 6 and 7, only under the assumption of absence of the double homozygous genotype in the offspring. Anyhow, it can be firmly established that in no case among the reciprocally compatible groups of a backcross family was there a ho ho obtained, *i. e.*, a genotype possible only at sporophytic pollen control in a family of that derivation. Whether this failure to appear is considered as being due to chance or to elimination of the double homozygotes following differential viability, difficulties are encountered. For instance, the conditions within the S_1 families 10 and 3 previously analysed, have given no direct indications of an elimination of the double homozygotes. The obvious under-representation of the double homozygotes demonstrated above to be due to mere chance, is still more improbable. The hypothesis of incompatibility in rye being purely sporophytically controlled in the pollen, has thus to be abandoned.

Parents of backcross family (S_1+×P)	Backcross family	Progeny from BCr+×BCr+
ho ho×het het $S_{1.1}Z_{3.4} \times S_{1.2}Z_{3.4} \rightarrow$	$S_{1.1}Z_{3.4}$ $S_{1.2}Z_{3.3}$ $S_{1.2}Z_{3.4}$ →	$S_{1.2}Z_{3.3}$ $S_{1.2}Z_{3.4}$ or $S_{1.1}Z_{3.4}$ $S_{1.2}Z_{3.4}$
ho het×het het $S_{1.1}Z_{3.4} \times S_{1.2}Z_{3.4} \rightarrow$	$S_{1.2}Z_{3.3}$ $S_{1.2}Z_{4.4}$ $2\,S_{1.2}Z_{3.4}$ →	$S_{1.1}Z_{3.4}$ $S_{2.2}Z_{3.4}$ $2\,S_{1.2}Z_{3.4}$

Scheme 3. Constitution of families derived from reciprocally compatible siblings within backcross families, when the backcrossed S_1 plant is ho ho and ho het, respectively. Gametophytic pollen control assumed (system I *a*, in text). Symbols according to Table 1.

There remains to be controlled whether the hypothesis of gametophytic pollen control is in agreement with data. Assuming a ho ho S_1 plant to be backcrossed to the het het parent plant, and then combining the reciprocally compatible genotypes of the backcross family, *viz. ho* het× het *ho*, the resultant progeny should segregate into two groups. When the S_1 plant is ho het, the reciprocally compatible genotypes will be ho_ahet and ho_bhet, and, accordingly, the corresponding number of groups should be three (see Scheme 3). In each of the four families studied three groups could be distinguished, thus indicating the backcrossed S_1 plants in all of these cases to be ho het. In an S_1 family derived from a double heterozygote and not affected by selection on the zygotic stage, ho het plants will be twice as frequent as the ho ho sibs, and thus a distribution equal to that of the present sample of S_1 sibs will not be improbable ($P=0{,}309$). Of the four S_1 plants thus tested and considered to be ho het, two gave some seed setting on selfing, and it will thus be possible in the S_2 and advanced families from selfing to test directly the genotypes of the S_1 plants in question.

C. Analysis in S_2- and S_3-families from S_1 plants considered on other evidence to be ho het

The two S_1 plants studied have been ♀ compatible to their parent plant. Strong evidence has already been presented for the latter to be heterozygous at two incompatibility loci and, therefore, the two ♀ compatible S_1 plants should be homozygous for at least one of these loci. On selfing an S_1 plant homozygous at both loci, the resultant S_2 family will be constituted by a single mating group. When homozygous at only

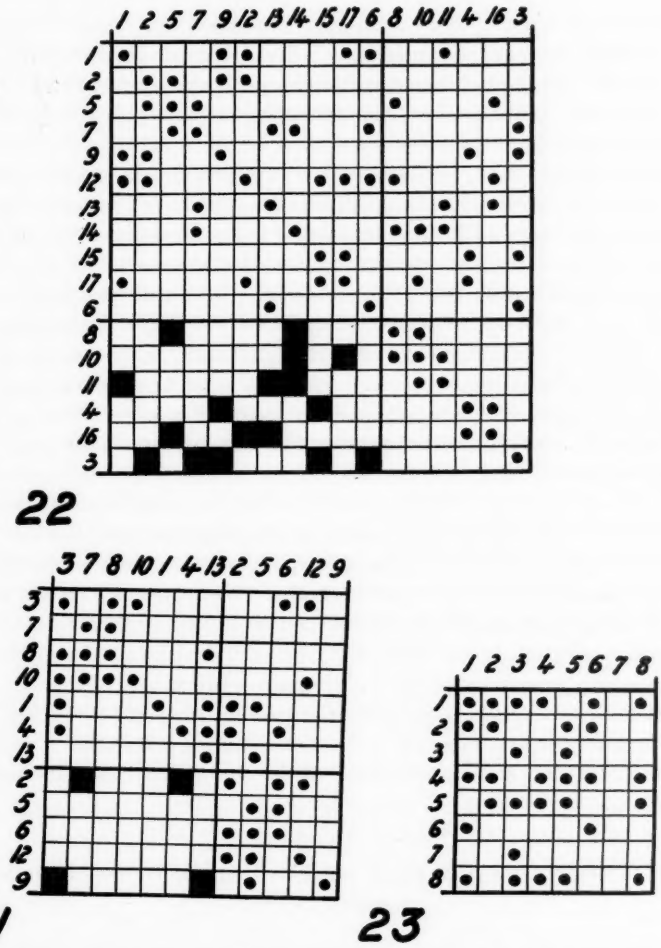


Fig. 8. Results from intercrosses within families derived from female compatible S_1 and S_2 plants selfed. Rows represent plants tested as females, columns, plants tested as males. Plants grouped according to their behaviour. Symbols according to Fig. 1. In family 23, plant 7 was male sterile and could not be tested as male.

one of the loci the selfed S_1 plant will segregate into three groups in its S_2 family, as should be expected for a monohybrid segregation (one ♀ incompatible heterozygous genotype, the other two being reciprocally compatible homozygous genotypes). Disturbances in this distribution are possible, due to the incompatibility reaction being of varying strength for different genotypes of the pollen, or resulting from differential viability of different zygotic genotypes.

Analysed are two S_2 families, both derived from clone 21, *i. e.*, families 21 and 22, respectively. The pollination patterns are given in Fig. 8. In both families, combinations may be compatible but never in both directions, only one-way; two groups can be distinguished, one of which is ♀ incompatible, the other ♂ incompatible. It is an important fact that more than one group can be distinguished, for it clearly demonstrates that the two S_1 plants selfed and giving rise to the S_2 families must have been heterozygous. As they have obviously been less heterozygous than the P plant, being ♀ compatible to the latter, the implication is arrived at that self-incompatibility is controlled here by more than one incompatibility locus. Assuming two such loci, the two S_1 plants must thus be considered as ho het, thereby corroborating the considerations based on completely different evidence in the foregoing section. Within the S_2 families in such a case, the ♀ incompatible group should be ho het whereas the ♀ compatible groups must be ho ho. (What has caused the absence of the second ho ho group expected cannot be definitely settled here, but gametic selection seems probable.) On selfing, double homozygous S_2 plants will give rise to an S_2 progeny with a single mating group. Within S_2 family 21, plant 2 was ♀ compatible with the other sibling group and thus considered to be ho ho. As expected, the S_2 family 23 resulting from selfing of plant 2, is completely intra-incompatible (Fig. 8).

3. Purely gametophytic or combined sporophytic-gametophytic pollen control?

The results so far obtained corroborate completely the hypothesis of purely gametophytic pollen control being exerted in rye. This hypothesis cannot thus be excluded when compared with the remaining hypothesis IV *a* (see section V: 1); at the most it will receive an alternative. The latter assumes two incompatibility loci, both necessary for a complete incompatibility reaction, one exerting the control of the pollen gametophytically, the other, sporophytically with independent

action of the alleles. According to the previous suggestion, the S locus is then assumed to be the »sporophytic» incompatibility locus, whereas the Z locus is the »gametophytic» one.

Starting from the nine genotypes possible in an S_1 family derived from a plant of the genotype $S_{1.2}Z_{3.4}$ and combining them in accordance with Scheme 1, the peculiar situation is now met with that in some combinations the pollen will react on the sporophytic scheme, *i. e.*, uniformly, disregarding its own genotype; in other combinations, again, on the gametophytic scheme permitting part of the pollen to function, and inhibiting the remaining part by means of an incompatibility reaction. When the pollen parent is homozygous for some allele at the »sporophytic» locus S, the phenotype of the pollen in terms of S will of course be equal to its genotype in S, and the pollen will, therefore, react following the gametophytic pattern of Scheme 1. Heterozygosity at the S locus, on the other hand, will be followed by the sporophytically determined reaction pattern of Scheme 1, in all cases except those where the pollinated plant, too, is heterozygous at the »sporophytic» locus. In such a case the »gametophytic» Z factor of the pollen will determine the reaction of the pollen.

When applying the given hypothesis to the data, the most critical evidence is borne out from the following consideration. For the argumentation to be followed more easily, the combinations of three genotypes, the progenies of which correspond to the categories 2 *B* and 4, respectively, in Scheme 1, are reproduced here.

2 *B*. $ho_a ho \times ho_a het$ (one-way compatible, only)
$S_{1.1}Z_{3.3} \times S_{1.1}Z_{3.4} \rightarrow S_{1.1}Z_{3.4}$ (on either hypothesis)

4. $ho_a het \times het\ het$ (one-way compatible only)

$$S_{1.1}Z_{3.4} \times S_{1.2}Z_{3.4} \rightarrow \begin{cases} \left(\begin{matrix} S_{1.1}Z_{3.3} \\ S_{1.1}Z_{4.4} \\ 2\,S_{1.1}Z_{3.4} \end{matrix}\right) \\ S_{1.2}Z_{3.3} \\ S_{1.2}Z_{4.4} \\ 2\,S_{1.2}Z_{3.4} \end{cases}$$

(Possible at sporophytic control at the S-locus)

The genotypes combined thus constitute a system of two successive steps of one-way compatibility ($S_{1.1}Z_{3.3}$ of course being only ♀ compatible to $S_{1.2}Z_{3.4}$). The ho het plant being homozygous at the S locus, the one-way compatible combination to a double homozygous sibling genotype will give only *one* group in the resultant progeny, whether the

control of the pollen is purely gametophytic or combined sporophytic-gametophytic. On the other hand, the progeny from the one-way compatible combination of the same ho het plant to the het het genotype will segregate into differing numbers of groups following the two alternatives for the pollen control; 3 groups being expected on the gametophytic scheme, and 6 on the sporophytic-gametophytic scheme.

In the S_1 family 10 (see Fig. 1) plants Nos. 8, 12, and 14 were already classified as ho ho, ho het, and het het, respectively. These three plants constitute a system of two successive steps of one-way compatibility, plant 8 being the bottom group, compatible only as female. As already demonstrated, the analysed offspring family 14 derived from the combination 8×12, contained a single group (see Fig. 3).

Assuming combined sporophytic-gametophytic pollen control, plant 12 being ho het must thus be assumed to be homozygous for one of the alleles at the »sporophytic» S locus, as family 14 would otherwise segregate into two groups. Following the scheme above, plant 8 may be denoted as $S_{1.1}Z_{2.3}$; plant 12, as $S_{1.1}Z_{2.4}$; and plant 14, as $S_{1.2}Z_{3.4}$. The combination 12×14 would thus segregate into 6 groups in the offspring. The corresponding offspring family 16 was already demonstrated to contain only three groups (see Fig. 4), this being the number expected on the scheme of purely gametophytic pollen control. The genotypes of family 16 have not yet been analysed more in detail, but judging from the previous analysis of genotypes in families of a similar derivation (from S_1 plants backcrossed to parent plant; see Table 6 *b*) the agreement between data observed and expected on the scheme of purely gametophytic pollen control above is not likely to be due to mere chance or to an elimination of certain zygotic genotypes. Sporophytic control of the incompatibility reaction to be exerted in the pollen thus seems to be excluded.

4. Conclusions

Cross-compatibility relationships in the two S_1 families tested required the assumptions (1) that the two presumed incompatibility loci are both necessary for a complete incompatibility reaction to be produced; and (2) that all the different incompatibility alleles constituting a certain genotype, affect the reaction of the pollen, naturally under the limitations arising from the time of this gene action to set in, whether on the sporophytic level, already, or limited to the gametophytic stage. From cross-compatibility relationships in a number of advanced families derived from S_1, it was demonstrated (1) that certain genotypes were lacking

though expected from an undisturbed segregation scheme and (2) that this absence was not a matter of mere chance but must be the indication of a functional disadvantage for certain pollen genotypes in the particular combination. This functional disadvantage speaks against sporophytic but in favour of gametophytic pollen control. In no case have data directly conflicted with the hypothesis of the pollen control being gametophytically exerted in the rye variety under investigation; almost in all respects is there a complete agreement.

Two incompatibility loci exerting gametophytic control of the pollen, will thus have to be presumed. It should be pointed out in this connection, that no allelic identities between the two loci can be proved to exist in the present material. Such identities would mean that the same allele could occur at either locus, so that the incompatibility reaction could be exerted between different loci; the frequency of cross-compatibility within an S_1 family will thereby be essentially reduced, and fewer incompatibility groups will be obtained. For instance, from selfing of a dihybrid but triallelic individual (*e. g.*, $S_{1.2}Z_{1.3}$; the suffices denoting the particular alleles irrespective of the loci) the resultant S_1 family will segregate into 9 genotypes, but only 5 incompatibility groups with 39,8 % cross-compatibility can be distinguished. It may be a matter of mere chance that only tetrallelic dihybrid parental clones have constituted the starting material of the analyses above; but the lack of allelic identities may be the indication of fundamental differences between the two loci; a critical decision, however, is not possible on the present material. It may be of significance here that the same conclusion of no allelic identities existing between the two loci, was arrived at also from the pooled S_1 data of all the 15 clones originally studied (LUNDQVIST, 1954).

VI. THE WAY OF COOPERATION BETWEEN THE INCOMPATIBILITY LOCI

1. The functioning ability of pollen with one factor matched in the style

The analysis so far accomplished has revealed that pollen with both factors matched in the style, is effectively inhibited by an incompatibility reaction, whereas pollen with only one of its two factors common to the style, is able to effect fertilization. As can be seen from Table 8, this fertilizing ability can be such as to bring about a seed setting equal to normal seed setting upon open flowering of materials inbred to an

TABLE 8. *Seed setting in fertile sib intercrosses within families derived from various cross combinations, the constituents of which are descendants of the same population plant, heterozygous at both incompatibility loci. All fertilizations effected by pollen with one of its factors common to the style (see text).*

Symbols according to Table 1.

Seed setting on open flowering in diploid S_1, 55—60 %, in diploid S_2, 45—50 % (LUNDQVIST, unpublished).

Parentage	Family number	Clone number	Year	Per cent seed setting																					n	$\bar{x}$
				0	5	10	15	20	25	30	35	40	45	50	55	60	65	70	75	80	85	90	95	100		
$S_1+ \times S_1-$	16	21	1955						1			1	1	2	1	3	7	5	2	3	3			1	30	64,0
$S_1+ \times P$	1	3	1954								2	2	1	2	1		4		3	5	1		1		22	61,8
»	7	16	»														5	3	4	1	2	1			16	70,9
»	17	21	»				1					2	1	1	2		1	1	1	2		2	2		16	62,8
»	18	»	»				1					2	1		1	1	3	6	4	6	2	3	3		33	69,6
BCr+ × BCr+	2	3	1955		5	2	1	1	1	1			1	2		2									16	25,9
»	9	16	»				3			1	1	2			1	1		1							10	35,0
»	19	21	»							1	1	1	2			1	2	1							9	48,1
»	20	»	»				1			1	2	1		2	1	2	1	2	3	4	1		1		22	59,5
Total					5	2	7	2	4	7	11	7	10	7	9	24	18	17	21	9	6	7	1		174	58,8

equivalent degree. The table shows seed setting in fertile sib intercrosses within families derived from cross combinations and segregating into three groups (genotypes). In all cases these families are traced back to a selfed plant heterozygous at both incompatibility loci, and the segregation does not thus involve more than four alleles, two at each locus. As double homozygous genotypes are not normally obtained upon crossing, all the sibling genotypes combined must be heterozygous at least at one of the loci, as has actually been demonstrated for some of the families in the foregoing sections. Thus in any intercross combination between the sibling genotypes there will be no pollen with none of its factors matched in the style; all fertilizations are effected by pollen with one of its factors common to the style. The resultant high percentage of seed setting, however, is no safe criterion that such pollen has in fact no functional disadvantage as it has functioned here without competition with foreign pollen. The fertilizing ability may be facultative and under natural conditions with open pollination, equal to effective incompatibility.

Such a functional disadvantage of pollen with one of its two factors matched in the style will be genetically indicated by disturbed segregation ratios in the offspring from suitable cross combinations where part of the pollen genotypes have none, the others at least one of their two factors common to the style. But such disturbances of the proportions expected for the different genotypes of the offspring, may be produced not only by a selective inhibition of pollen tube growth but also by a direct elimination (differential viability) of genotypes on the zygotic or the gametic level. It will be necessary, therefore, first to look for the occurrence of eliminations of that kind.

The parental combinations in Table 8 all correspond to the categories 4 *E* or 5 *F* of Scheme 1. Gametophytic pollen control is postulated. As can be seen from the scheme, the resultant offspring under ideal conditions, without direct eliminations of genotypes on either the gametic or the zygotic level, will in all cases segregate into equal proportions of double heterozygotes and single heterozygotes, the latter being represented by two genotypes. The het het plants are ♀ incompatible, whereas the ho het genotypes are ♀ compatible when combined with other genotypes within the family. Direct genotypic elimination on the gametic level, either on the female or on the male side will cause no change of the total ratio het het : ho het in the offspring, whereas the ratio 1 : 1 between the ho het genotypes may be changed. Only when occurring at the same time on the male side as well as on the female

TABLE 9. *Segregation within the families of Table 8 into female incompatible and female compatible plants. Expected ratio = 1 : 1.*

a. Individual data.

Clone number	Family number	Parent genotypes	Category of scheme 1	A ♀ incomp. plants	B ♀ comp. plants	Probability of the observed deviation from 1:1 ratio A/B
3	2	ho_ahet × ho_bhet	5 F	3	3 + 3	260/512 = 0,51
16	9	» »	»	2	3 + 2	58/128 = 0,45
21	19	» »	»	2	1 + 4	58/128 = 0,45
»	20	» »	»	6	3 + 3	4096/4096 = 1,00
3	1	ho het × het het	4 E	9	2 + 3	6946/16384 = 0,42
16	7	» »	»	6	2 + 1	260/512 = 0,51
21	16	» »	»	4	4 + 3	1124/2048 = 0,55
»	17	» »	»	4	4 + 3	1124/2048 = 0,55
»	18	» »	»	6	5 + 3	12952/16384 = 0,79
Total				42	52	$\chi^2 = 1{,}064$ $0{,}5 > P > 0{,}3$

b. Pooled data.

Clone number	A ♀ incomp. plants	B ♀ comp. plants	χ^2	d. f.	P
3	12	11	0,043	1	0,9 — 0,8
16	8	8	0,000	1	1,0
21	22	33	2,200	1	0,2 — 0,1
Sum of χ^2			2,243	3	0,7 — 0,5
Pooled data			1,064	1	0,5 — 0,3
Heterogeneity			1,179	2	0,7 — 0,5

side of the parent plants, will a direct elimination of gametic genotypes affect the ratio 1 : 1 between het het plants and ho het plants in the offspring. Elimination of genotypes on the zygotic level will have the same effect; it will thus be difficult to decide between the two alternatives, but for the present purpose it will be enough to decide if direct elimination of certain zygotic genotypes is made probable at all or not.

The segregations within the nine families of Table 8 are compared in Table 9. The individuals are grouped according to female reaction, compatible and incompatible, respectively; the expected ratio being 1 : 1. Within the ♀ compatible (ho het) category, the distribution between the two genotypes possible has been entered. As can be seen from the table for the whole material — 94 plants studied — the segregation ratio obtained between the het het and the ho het categories is in a good agreement with the proportions expected. Between the three parental clones there are no statistically significant differences in the segregation ratios, and within the individual families the agreement with the expected proportion 1 : 1 is also good. There is, thus, obtained no indication that differential viability of zygotic genotypes should be a factor essentially affecting the segregation ratios.

Within the ho het category the distribution between the two genotypes possible, is fairly even in all nine cases. The material is rather meagre, admittedly, but from the data no indication is obtained of the fertilizing ability differing in different genotypes of pollen with one factor common to the style.

Disturbances, if any, affecting the expected segregation ratios between the incompatibility groups, thus might be the indication of a weak functional disadvantage of pollen with one of its two factors common to the style; direct eliminations of genotypes, either on the gametic or on the zygotic level probably being of secondary importance in this connection.

In order to test critically the possible occurrence of such a weak functional disadvantage of pollen with one incompatibility factor common to the style, pollen from heterozygotes was applied to double homozygous sib plants. The double homozygous plant will produce gametes of only one genotype, and thereby the complications are avoided arising from a possible elimination of certain egg genotypes in a heterozygous female plant. At the same time each genotype in the offspring will result in a single way, from the functioning of a particular pollen genotype. Thus, double heterozygous offspring obtained from the pollinated double homozygous plant, can be explained only from the functioning

of pollen with no factor common to the style, whereas homozygosity at one or both incompatibility loci will be obtained in the offspring when the functioning pollen has one or both of its two factors matched in the style. The frequency of the different zygotic genotypes in the offspring will thus constitute a direct measure of the fertilizing ability of the different pollen genotypes.

In Scheme 1, the categories 2 *C* and 3 *D* constitute such test combinations. 2 *C* corresponds to the reciprocally compatible combination ♀ $S_{1.1}Z_{3.3} \times$ ♂ $S_{2.2}Z_{3.4}$; and 3 *D*, to the one-way compatible combination ♀ $S_{1.1}Z_{3.3} \times$ ♂ $S_{1.2}Z_{3.4}$. The former combination may be realized from sib intercrosses in an S_1 family derived from a dihybrid parent plant; the latter, under the same conditions, and from backcross to the parent plant. The two categories are illustrated in the scheme below:

2 *C*. $ho_a ho \times ho_b het$

Parents	$S_{1.1}Z_{3.3}$	$\times S_{2.2}Z_{3.4}$
Gametes	S_1Z_3	S_2Z_3 S_2Z_4
Offspring	$S_{1.2}Z_{3.3}$	$S_{1.2}Z_{3.4}$

3 *D*. ho ho × het het

Parents	$S_{1.1}Z_{3.3}$	$\times S_{1.2}Z_{3.4}$
Gametes	S_1Z_3	(S_1Z_3) S_1Z_4 S_2Z_3 S_2Z_4
Offspring	$(S_{1.1}Z_{3.3})$	$S_{1.1}Z_{3.4}$ $S_{1.2}Z_{3.3}$ $S_{1.2}Z_{3.4}$

In the pollen genotypes, the matched factors are in bold type; in each category one of the genotypes has none of its two factors common to the style. Fertilization by such pollen will in both cases result in double heterozygous plants, whereas the remaining ho het plants result from fertilization by pollen with one factor matched in the style. Pollen with both factors matched in style is fully incompatible, and double homozygous plants are not thus to be expected in the offspring. If the double heterozygous genotype is more frequent than expected from the scheme above, this indicates a functional advantage of the corresponding fertilizing pollen with no factor matched in the style.

Of interest in this connection are two families, Nos. 12 and 15, both derived from intercrosses between S_1 siblings in family 10 from the parent clone 21. In both cases the female sib plant was on other evidence considered to be double homozygous.

Family 12. — As demonstrated above (*cf.* p. 316) this family was obtained from the combination $ho_a ho \times ho_b het$, the combination thus corresponding to category 2 *C* in the scheme above. The pollination pattern is shown in Fig. 4. Two one-way inter-compatible groups are

obtained, out of which the ♀ incompatible group must obviously be het het resulting from fertilization by pollen with no factor common to the style, whereas the other, ♀ compatible, group must be ho het and result from functioning pollen with one of its two factors matched in the style. Of the 12 plants tested, 3 are het het and 9 are ho het. The observed deviation from the ideal distribution 1 : 1 is not significant ($\chi^2=3{,}0$; $0{,}1 > P > 0{,}05$). A functional disadvantage of the pollen with one factor matched in the style is not indicated at all, and pollen of that kind has, on the contrary, functioned at least as frequently as has the other category with no factor common to the style.

Family 15. — This family is derived from a combination ho ho × het het (*cf.* p. 316); the combination thus corresponding to category 3 *D* above. The pollination pattern is shown in Fig. 4. Three groups are obtained, one of them being ♀ incompatible but ♂ compatible on the other two, reciprocally compatible groups. These compatibility relationships indicate that the ♀ incompatible group must be het het, the other two, ho het. Three of the four genotypes in the pollen have thus functioned, one of them having no factor, the other two, one factor, common to the style. The three groups are each represented by two individuals, this corresponding exactly to the proportions expected in the scheme. Here, too, no functional disadvantage is indicated to arise from one factor, as compared to no factor, common to the style.

2. Are there indications of dominance allelic interaction in the pollen?

Pollen with one factor matched in the style has, thus, in neither case suffered a reduction of its fertilizing ability. The material tested is small, however, and one possible explanation might be that the other factor of the pollen, not matched in the style, has in the present cases acted in a dominant way. (Further data, considered later, will show that it can not be the matter of a positive promotion of pollen tube growth produced by unlike factors in pollen and style and always dominating the positive inhibitory reaction by like factors.) The proposed dominance interaction will require, as a corollary, the further assumption that pollen of the present genotype is fully incompatible when the dominant factor is matched in the style, the other factor being of no significance in that connection. Such a dominance allelic interaction in the pollen must be an accidental occurrence. All the evidence previously presented, primarily the high frequency of cross-compatibility in the S_1 families, and the uniform reaction pattern of inter-incompatible plants will re-

quire a regular and equal contribution by the two factors of the pollen to the incompatibility reaction. This conclusion is not consistent with regular dominance interaction relationships between the alleles of the pollen as proposed above, for such an interaction would result in an intra-incompatible group including more than one genotype. This in its turn might result in an ununiform reaction pattern within the group and in any case in a more or less lowered frequency of cross-compatibility between siblings of the family, the details depending on the particular dominance relationships between the two loci and the particular alleles. Space does not permit here more details in that connection. It seems highly improbable, however, that the two families tested here would prove the existence of dominance interactions between alleles of the pollen, excluded on the evidence previously referred to. The following considerations may be added.

In the four pollen genotypes possible from the dihybrid parent clone P 21 (with the genotype arbitrarily denoted as $S_{1.2}Z_{3.4}$) four dominance relationships between the two loci can be established at a time, constituting a complete system of dominance relationships. In family 12 one of these dominance relationships would thus have appeared; in family 15, two of them (see the scheme above!). Thus, at least two, and possibly three dominance relationships between the loci would have been established in clone 21.

Considering first the relationships indicated by family 15, the double homozygous mother plant No. 8 may be denoted as $S_{1.1}Z_{3.3}$, and the double heterozygous father plant No. 14, as $S_{1.2}Z_{3.4}$. Accordingly, the pollen S_1Z_3 must be inhibited, whereas S_2Z_4 with no factor common to the style, is uninhibited. S_1Z_4 and S_2Z_3 have one factor common to the style; they have both shown no inhibition, and according to the hypothesis of dominance allelic interaction in the pollen, Z_4 should be dominant to S_1; and S_2, to Z_3. Two dominance relationships, $S_1 \leftarrow Z_4$ and $S_2 \rightarrow Z_3$ would thus have been established.

In family 12 the two pollen genotypes from the ho het father plant No. 2 have functioned equally well although one of them had one factor common to the style. Assuming the two dominance relationships presumed above to be the only ones established in clone 21, it will be found that the double homozygous mother plant No. 16 of family 12 must be supposed to be $S_{1.1}Z_{3.3}$. Pollen of the »dominance» genotypes S_1Z_4 or S_2Z_3, respectively, will function thanks to a dominance interaction effect, when one of the pollen factors is matched in the style, the matched factor being the recessive S_1 or Z_3. For these conditions

to be fulfilled, the genotype of the mother plant must be $S_{1.1}Z_{3.3}$. However, as can be seen from Fig. 1, the genotype of plant 8 already denoted as $S_{1.1}Z_{3.3}$, cannot be identical with that of plant 16. Thus it would be necessary to assume still another dominance relationship in clone 21, established within family 12. Three dominance relationships established out of four possible will not necessarily result in a conspicuous lowering of the frequency of cross-compatibility within the S_1 family or in inter-incompatible plants giving a highly ununiform compatibility reaction pattern with common third tester plants. In the S_1 family 10, out of 48 cross combinations, 25 or 52,1 % were compatible. The expected value for cross-compatibility being 60,9, the deviation observed is not statistically significant ($\chi^2=1{,}542$; $0{,}3 > P > 0{,}2$), but as the frequency is lower than expected, it may be justifiable here to explain more in detail why the hypothesis of dominance allelic interaction in the pollen operating in clone 21 will be untenable and has to be rejected.

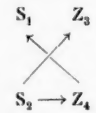
In the two dominance relationships already presumed, *viz.* $S_2 \rightarrow Z_3$, and $S_1 \leftarrow Z_4$, the action of S_2 in relation to Z_3 is established, and the same applies to S_1 relative to Z_4. The supposed third dominance relationship may thus apply to S_2 in relation to Z_4, or S_1 in relation to Z_3. It is not unimportant if this third dominance relationship applies to the two factors dominating above, *viz.* S_2 and Z_4, or to the two recessive ones, *viz.* S_1 and Z_3. Within each of these relationships, the specific relations between the alleles is of no account. In the following, the two alternatives will be considered separately.

Assuming S_2 to be dominant to Z_4, the combination ho het by the double heterozygote $S_{1.2}Z_{3.4}$ (sib plant or parent clone) will segregate in the offspring into three groups and three genotypes only when the ho het female plant is $S_{1.1}Z_{3.4}$. The three remaining ho het genotypes under the same conditions will give only two groups and two genotypes in the offspring (Scheme 4 *a*). The offspring family from the combination ♀ $S_{1.1}Z_{3.4} \times$ ♂ $S_{1.2}Z_{3.4}$ segregates into $S_{1.2}Z_{3.3}$, $S_{1.2}Z_{4.4}$, and 2 $S_{1.2}Z_{3.4}$. In the following generation derived from the sib intercross $S_{1.2}Z_{3.3} \times S_{1.2}Z_{4.4}$ and reciprocally, only two groups and two genotypes are obtained. Within clone 21 without exception all families from ho het S_1 plants as female backcrossed to the parent clone or intercrossed to double heterozygous siblings have segregated into three groups (families 16, 17, and 18). Intercrosses between the inter-compatible ho het genotypes of families 17 and 18, respectively, in their turn also resulted in an offspring segregating into three groups (families 19 and 20). The

$S_{1.2}Z_{3.4}$

	S_1Z_3	$S_1Z_④$	$S_②Z_3$	$S_②Z_4$
$S_{1.1}Z_{3.4}$			$S_{1.2}Z_{3.3}$ $S_{1.2}Z_{3.4}$	$S_{1.2}Z_{3.4}$ $S_{1.2}Z_{4.4}$
$S_{2.2}Z_{3.4}$	$S_{1.2}Z_{3.3}$ $S_{1.2}Z_{3.4}$			
$S_{1.2}Z_{3.3}$		$S_{1.1}Z_{3.4}$ $S_{1.2}Z_{3.4}$		
$S_{1.2}Z_{4.4}$	$S_{1.1}Z_{3.4}$ $S_{1.2}Z_{3.4}$			

a.



$S_{1.2}Z_{3.4}$

	$S_①Z_3$	$S_1Z_④$	$S_②Z_3$	S_2Z_4
$S_{1.1}Z_{3.4}$			$S_{1.2}Z_{3.3}$ $S_{1.2}Z_{3.4}$	$S_{1.2}Z_{3.4}$ $S_{1.2}Z_{4.4}$
$S_{2.2}Z_{3.4}$	$S_{1.2}Z_{3.3}$ $S_{1.2}Z_{3.4}$			
$S_{1.2}Z_{3.3}$		$S_{1.1}Z_{3.4}$ $S_{2.2}Z_{3.4}$		$S_{1.2}Z_{3.4}$ $S_{2.2}Z_{3.4}$
$S_{1.2}Z_{4.4}$				

b.

Scheme 4. Genotypes segregating from the combination ♀ ho het (four different genotypes) by ♂ het het under the assumption of gametophytic pollen control with three dominance interaction relationships established between the loci. Pollen of four genotypes obtained from the het het plant; each of them with a separate pollination column in the scheme. Dominant factors encircled. Dominance relationships to the right.

dominance relationships $S_2 \rightarrow Z_4$ (and consequently $S_2 \leftarrow Z_4$, too) are thus not possible.

Assuming S_1 to be dominant to Z_3, the combination ho het by the related double heterozygote $S_{1.2}Z_{3.4}$, will segregate in the offspring into three groups and three genotypes only when the ho het female plant is $S_{1.2}Z_{3.3}$ (see Scheme 4 *b*). From $S_{1.1}Z_{3.4}$ also three offspring genotypes are obtained in the corresponding offspring; however, two of them, *viz.* $S_{1.2}Z_{4.4}$ and $S_{1.2}Z_{3.4}$, are mutually incompatible and react uniformly within the family, and thus only two groups in the family can be distinguished here. The offspring family from the combination ♀ $S_{1.2}Z_{3.3} \times$ ♂ $S_{1.2}Z_{3.4}$ segregates into $S_{1.1}Z_{3.4}$, $S_{2.2}Z_{3.4}$, and 2 $S_{1.2}Z_{3.4}$. In the following generation

derived from the sib intercrosses $S_{1.1}Z_{3.4} \times S_{2.2}Z_{3.4}$ and reciprocally, only two groups can be distinguished. Thus, even the last dominance relationship assumed, *viz.* $S_1 \rightarrow Z_4$ (and conversely), must be rejected.

3. Conclusions

In order to explain why pollen with one factor matched in the style has exhibited no functional disadvantage as compared to pollen with no factors matched, the assumption of dominance allelic interaction in the pollen has turned out inadequate. In this connection, family 15 is of special importance, since data here indicate that pollen of the genotypes S_1Z_4 and S_2Z_3 did function equally effectively on female plants $S_{1.1}Z_{3.3}$; thus, it did not matter to which locus the matched factor belonged, and no functional disadvantage resulted in either case. Experiments are carried on to see if this observation may have a wider application. The fact that the compatibility reaction has not been noticeably affected by one factor of the pollen matched in the style may be due to allelic interaction in the pollen resulting in mutual weakening of the reaction. If there was a critical threshold strength of the reaction below which all pollinations appeared fully compatible, the attainment of this threshold value, only from the added reactions of two matched, mutually weakened factors, would be conceivable. Data obtained from an analysis of the incompatibility relationships in tetraploid rye has, however, demonstrated that out of the four factors carried here in the pollen, not all will have to be matched in the style, to produce a complete incompatibility reaction (LUNDQVIST, unpublished). Therefore it does not seem very probable that the observed inefficiency of a single factor common to pollen and style could result from the existence of a threshold value of the incompatibility reaction. At the same time one has to reject definitely the alternative hypothesis of a positive promotion of pollen tube growth by unlike factors in pollen and style always dominating the positive inhibitory reaction by like factors. The combined evidence points to the view that the two genetically separate loci behave as a physiological unity and that the specific incompatibility substances result from interactions between alleles at the two loci.

VII. DISCUSSION

The postulated system is rather unusual and it will therefore be important to establish confidence in the experimental technique. In 1954

the crossing program comprised 152 plants in 558 cross combinations, involving more than 20000 pollinated flowers; only one of the combinations (in family 3) gave an inexplicable result. In 1955 were tested 113 plants in 462 cross combinations involving more than 18000 pollinated flowers. No inexplicable results were obtained, the few, not clearly unambiguous ones (about 1 % of the total number of combinations) being satisfactorily explained from reduced vitality, unfavourable pollination conditions, or pseudo-fertility. In all, 23 families were analysed, and in five of them the number of segregating groups did not conform exactly to the number expected, a lower number being obtained. In three of these families, however, some of the test combinations necessary for a complete segregation analysis are lacking (families 3, 8, and 10). The two remaining families do not conflict directly with the proposed hypothesis as the lack of one of the groups expected in the S_2 families 21 and 22 may be due to differential viability of zygotic genotypes or to a stronger incompatibility reaction of one of the pollen genotypes. The reaction patterns within the other families conform to expectation; in no case was the introduction of auxiliary hypotheses necessary. A defect in the argument is, however, that the genotypes represented within the different families are determined only indirectly by inference, the direct testing on conserved genotypes of known constitution not being possible owing to the difficulty to prevent the annual rye from dying after completion of flowering.

In the comparatively uncomplicated material constituted by an S_1 family the segregation will permit a simpler and more clear cut analysis; but other complications may arise from selfing, leading to wrong conclusions. Disturbed segregations may result from different pollen genotypes exhibiting differing disposition towards pseudo-fertility in the self-pollinated style, these differences depending either on varying rigour of the incompatibility reaction (*cf.* EAST, 1934 a and b) or on varying frequency of temporary complete loss of allelic activity, as suggested for *Oenothera organensis* (LEWIS, 1951). However, the results in the advanced families derived from crossing are quite clear cut and corroborate the conclusions drawn from the families from selfing. Contaminants resulting from the strong selective advantage in the self-pollinated style for mutations to new constructive self-incompatibility alleles may also be possible. So far, no critical evidence for such constructive new mutations to occur at the incompatibility locus has been obtained even in the intensively studied materials of *Oenothera organensis* and *Prunus avium* (LEWIS, 1949 b, 1951, 1954 a). In the present

material also, the evidence from backcrosses $P \times S_1$ speaks against the occurrence of such contaminants; all the 38 S_1 individuals tested, were male incompatible on the parent plant (LUNDQVIST, 1954). Starting, on the other hand, from a combination between unrelated individuals, the further analysis would become considerably complicated; from dihybrid parent genotypes with no alleles in common, 16 different genotypes are possible in the offspring, all inter-compatible in all directions, even with the parental individuals.

It should be pointed out here that the incompatibility system outlined above may display features previously held up as especially characteristic of homomorphic systems with sporophytic control of the pollen (*cf.* LEWIS, 1954 a; BATEMAN, 1954); these features would be difficult to interpret without information about the number of incompatibility loci operating, or a strict analysis of pollen tube growth. Combinations such as $♀ S_{1.1}Z_{3.4} \times ♂ S_{1.2}Z_{3.4}$, to be sure, may be relatively rare in a cross-breeding population, but the genotypes both seem to be normal constituents of such a population. The resultant F_1 will segregate into three groups ($S_{1.2}Z_{3.3}$, $S_{1.2}Z_{4.4}$, $S_{1.2}Z_{3.4}$) that are not inter-compatible in all directions ($S_{1.2}Z_{3.4}$ failing as female). Backcross to the seed parent will sometimes be incompatible ($♀ S_{1.2}Z_{3.4} \times ♂ S_{1.1}Z_{3.4}$). These are all features that may be found in biparental families when pollen control is sporophytic, but will not be possible at one incompatibility locus operating on the gametophytic scheme of pollen control. Only a continued analysis will reveal that a particular intra-incompatible group will never contain more than one genotype, thus giving the first evidence of the novel type of pollen control actually exerted.

The main characteristic of the identified system, is the fact that pollen with one factor matched in the style is able to function without any noticeable functional disadvantage resulting from the matched factor. As pointed out before (LUNDQVIST, 1954), an incompatibility system, where the incompatibility alleles are apportioned to two different series (loci), and where both factors of the pollen have to be matched in the style to produce a full incompatibility reaction, will mainly involve an increased stability against loss of incompatibility alleles due to random drift; besides an increase of general cross-compatibility will follow. At a great number of incompatibility alleles under the conditions given above, the number of incompatibility loci will have but little effect on cross-compatibility. It is conceivable, however, that the effect of one factor of the pollen matched in the style could be sufficiently strong to put such pollen at a disadvantage when competing with pollen with no

factor common to the style. The stronger this functional disadvantage will turn out, the more will be lost of the previous advantageous effect on cross-compatibility associated with a biserial incompatibility system. In the extreme case, identity at only one of the loci being sufficient to produce a full incompatibility effect, cross-compatibility on the biserial system will be so restricted that the monoserial system will now considerably less interfere with the interbreeding of the population (*cf.* BATEMAN, 1952, Table 2). As pointed out by BATEMAN, this general reduction of cross-compatibility will put the biserial incompatibility system at a selective disadvantage.

No combinations between unrelated plants were analysed, and thus the character of the two loci could not be settled definitely, whether multiallelic or not. It is important here that the three more critically analysed clones were all dihybrid, and that the pooled data of the 15 clones originally studied conform quite well to the conclusion that they were all heterozygous at both incompatibility loci (LUNDQVIST, 1954). No indication being obtained of a functional disadvantage of pollen with one factor matched in the style, this prevalent heterozygosity seems primarily to imply at least a moderate number of incompatibility alleles at both loci occurring in the population. Assuming the alleles to be equally frequent and equally apportioned to the two loci with n alleles at each, the frequency of double heterozygotes in the population will be $\frac{(n-1)^2}{n^2}$, and of single heterozygotes, $\frac{2(n-1)}{n^2}$. Double homozygotes are not normally obtained. The ratio of double heterozygotes to singly heterozygotes will thus be $\frac{n-1}{2}$. Thus, in a sample of 15 individuals from a population with 10+10 different alleles, the probability of a distribution equal to all 15 being double heterozygotes, is 0,09, and at 25+25 alleles the corresponding probability is 0,62; the ratio of double heterozygotes to singly heterozygotes in the former case being 4,5, in the latter case, 12.

The question has already been treated at some length whether the occurrence of two incompatibility loci in rye might be taken to indicate duplications to exist in the rye genome (LUNDQVIST, 1954). So far, no other genetical data have appeared indicating rye to be a partial polyploid. It should be pointed out here that besides the *Gramineae*, the only case of more than one incompatibility locus being established fairly positively in a homomorphic diploid Angiosperm, seems to be *Beta vulgaris* (OWEN, 1942); perhaps also *Abutilon hybridum* (SEARS, 1937).

Other cases of more than one locus being suggested, have either turned out to be associated with polyploidy (LAWRENCE, 1930) or have been explained as more or less complicated cases of sporophytically controlled incompatibility (BATEMAN, 1955). The sugar beet has originated relatively recently, *viz.* in the 1850's being probably obtained in the segregants resulting from a spontaneous crossing between previously cultivated beets and the wild beet, *Beta maritima* (RASMUSSON, 1951). It is of considerable interest in this connection, that the two loci in *Beta vulgaris*, seem to be duplicate with allelic identities between the loci, so that the incompatibility reaction may be exerted between different loci (OWEN, *l.c.*). In the present rye material, no such allelic identities between the loci could be observed. Either this may be due to mere chance, the material investigated not being particularly extensive, or such identities might have been at a selective disadvantage resulting from reduced cross-compatibility, thus being eliminated; but it may also indicate a more fundamental difference between the two loci. Two facts are of importance in this connection:

(1) The incompatibility reaction in rye when produced must be considered to be very strong with poor germination of the pollen on the incompatibly pollinated style (SEARS, *l. c.*), but at the same time no functional disadvantage of the pollen has been observed to result from one factor being common to pollen and style. Combined evidences from the diploid and the tetraploid stage have indicated that this may not be due to interactions in the pollen between the two loci resulting in dominance relationships or mutual weakening, nor to a dominating positive promotion of pollen tube growth by unlike factors. Indicated is a regular cooperation between the loci, both involved together in the incompatibility reaction, and the specific incompatibility substances thus resulting from interactions of the two loci. In other words, the two loci do not function in spite of but through each other. So far, such interaction between loci in the case of incompatibility factors has not been observed in Angiosperms but has been made probable in the tetrapolar fungus *Schizophyllum commune* (PAPAZIAN, 1951); here one at least of the two incompatibility factors proved to consist of two linked loci at 4 units apart, matings between mycelia identical at only one of these two linked loci being compatible.

(2) The incompatibility reaction in homomorphic Angiosperms seems to be an immunity reaction between pollen and style, between antigens and antibodies, as especially made probable through serological tests by LEWIS (1952). A compound nature of the oppositional S locus has

been demonstrated for two species of *Oenothera*, two of *Trifolium*, and one of *Prunus*; one of the unities controlling the reaction of the pollen, the other unity, the reaction of the style, and both capable of independent mutation (LEWIS, 1949 b; LEWIS and CROWE, 1953; LEWIS, 1954 a). Recently, it was suggested by LEWIS (1954 b) that pollen contains a substance necessary for the pollen tube to develop at all. In self-sterile species »specificities have been imposed on this substance by haptene groups produced by the S alleles. In the style the S alleles produce preformed antibodies to the pollen substance». CROWE (1955) has then presented evidence from some species of *Oenothera* of such a substance occurring in the pollen and produced by a third unity at the S locus. Here each allele is probably producing a specific growth substance carrying superimposed specific haptene groups from the S allele and in this stage reacting incompatibly with preformed antibodies in the style.

In rye also one of the two incompatibility loci has given evidence of a compound structure (LUNDQVIST, unpublished). Self-compatible spontaneous mutants, obtained from selfing of self-incompatible plants, might be male compatible but female incompatible with their parent plant. The specificity of the pollen was thus lost, whereas the action in the style was retained. It is not yet known how the second locus will behave in this respect; it is conceivable, however, that this locus might be responsible for the production of the growth substance of the pollen. It should be pointed out here that rye counts among the species mentioned by LEWIS (1954 b) as giving evidence of the existence of the pollen growth substance suggested. The tripartite locus in *Oenothera* would then be equal to the combination of the two separate loci in rye.

Naturally, it is quite conceivable that the two loci are at least analogous, selection having had an opportunity here to affect allele interaction in the pollen so as to render it consistent with a normal functioning of the incompatibility mechanism. The perfect self-incompatibility in autotetraploid rye speaks in favour of this alternative. For after chromosomal doubling most diploid species with gametophytically controlled self-incompatibility, will display tendencies to a breakdown of the incompatibility mechanism, this breakdown being due to the fact that the incompatibility alleles, prior to the chromosomal doubling always occurring singly in the pollen, will now occur in pairs in the pollen and thus have an opportunity to interact, the interaction sometimes resulting in mutual weakening of the alleles (LEWIS, 1947). The complete or almost complete lack in autotetraploid rye of these ten-

dencies to a breakdown of the incompatibility mechanism (LUNDQVIST, 1947, and unpublished) could thus be explained as resulting from a previous selection at the diploid level, already, for the ability of the incompatilibity genes to work efficiently in pairs on the male side. [It will, however, be necessary to study if pollen tube growth in incompatibly pollinated tetraploid rye is affected, as was the case in autotetraploid *Oenothera organensis*, where the weakening of the incompatibility reaction was indicated mainly by an increased pollen tube growth, not affecting seed setting (LEWIS, 1943, 1947.)] Such a selective balancing of the interaction between the incompatibility genes would also be possible when pollen control is sporophytic, because the genes will then regularly occur in pairs at their active stage. As expected here, self-incompatibility seems to be unaffected by polyploidy (GERSTEL, 1950; *cf.* BATEMAN, 1955). Experiments are carried on to test critically the character of the two loci in rye.

That the biserial incompatibility system observed in the rye variety analysed, is no accidental, isolated case, will be evident from a similar genetical analysis of self-incompatibility in another grass species, *viz. Festuca pratensis* HUDS., the Meadow Fescue, where a similar system seems to be operating (LUNDQVIST, 1955). A wide distribution of such an incompatibility system within the *Gramineae* may be indicated by cases of polyploid grass species with perfect self-incompatibility. It may be of significance that the grasses and *Beta vulgaris*, the only cases so far observed of more than one incompatibility locus operating in a diploid, are wind pollinated.

SUMMARY

(1) A genetic analysis of self-incompatibility in rye was performed in three selected population plants belonging to the Swedish commercial variety »Steel-rye» and displaying different strength of the incompatibility reaction. Seed setting was studied after sib intercrosses within offspring groups derived by various ways of inbreeding from the three selected parental plants.

(2) S_1 progeny groups from two of these plants were studied. In each, at least six mating groups could be distinguished. More than one incompatibility locus must thus be operating, two being presumed.

(3) Negative correlation between male and female reaction, female incompatibility of the parent plant with its S_1 progeny, and absence of true self-compatibility segregating, indicate sporophytic incompatibility control in the style with independent action of the alleles.

(4) Data from the two S_1 families indicate that the two presumed incompatibility loci are both necessary for a complete incompatibility reaction to be produced, and that all the different incompatibility alleles constituting a certain genotype affect the reaction of the pollen.

(5) Different oppositional systems for the incompatibility control in the pollen are tried in model experiments. Three of them are compatible with the previous S_1 data, one assuming gametophytic incompatibility control at both loci, one, sporophytic control, and the last, gametophytic control at one locus, sporophytic control at the other locus.

(6) Data from advanced families derived from S_1 demonstrate that genotypes are sometimes lacking though expected from an undisturbed segregation scheme, and that this absence is not fortuitous but indicates a functional disadvantage for certain pollen genotypes in the particular combination. The two alternatives that consider sporophytic pollen control are both analytically eliminated. Data are satisfactorily explained by two incompatibility loci exerting a gametophytic control of the pollen. Both loci are most probably multiallelic.

(7) No allelic identities between the two loci are indicated in the present material.

(8) Pollen with both factors matched in the style, is effectively inhibited and can not normally effect fertilization in open competition. Pollen with one factor matched in the style, exhibits no functional disadvantage as compared to pollen with no factors matched. It does not matter which locus the matched factor belongs to; no functional disadvantage results in either case.

(9) This lack of a functional disadvantage resulting from one factor of the pollen matched in the style, could not be explained by various relationships of dominance allelic interaction in the pollen. Nor is it possible to explain it from a positive promotion of pollen tube growth by unlike factors in pollen and style always dominating the positive inhibitory reaction by like factors.

(10) The two genetically separate loci behave instead as a physiological unity, and the specific incompatibility substances seem to result from interactions between alleles at the two loci.

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CYTOLOGY, EVOLUTION AND ORIGIN OF THE ANEUPLOID SERIES IN THE GENUS CAREX

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I. INTRODUCTION

THE genus *Carex* unlike the majority of plant genera contains a long aneuploid series. The mechanisms of speciation in genera with polyploidy have been the subject of many investigations (*e. g.* BABCOCK, 1942, 1947, *etc.*; MANTON, 1950, and VALENTINE, 1950), but the mechanism involved in the development of species through aneuploidy has been comparatively neglected, although a classification into three different naturally occurring types was attempted by STEBBINS in 1950. It therefore seemed of interest to investigate certain species of *Carex* with this end in view.

Carex, it is well known, presents considerable technical difficulties to the cytologist, and attempts to obtain reliable chromosome counts and other information from somatic mitosis have in most cases failed, although the chromosome numbers recorded by HEILBORN studying meiosis by means of the embedding and sectioning methods have proved remarkably accurate. By a slight modification of the aceto-carmine squash technique satisfactory preparations of meiosis in the pollen mother cells, and mitosis in the pollen grains have been obtained, and these have provided all the numbers given in Tables 1 and 2. In view of the cytological difficulties and uncertainty of identifications in many published accounts, the opportunity has been taken to make counts on material of known origin of all available species. Specimens from the counted plants are preserved in the Herbarium of University College, Leicester.

II. A BRIEF SURVEY OF PREVIOUS WORK

The cytology of this genus has attracted many workers during the past fifty years. The reasons for this are probably varied, but it is certainly in part due to the fact that the *Cyperaceae* has been shown to

TABLE 1. *Chromosome numbers in the genus Carex.*

Subgenus I. Carex	Species	Haploid chromosome number	Number of plants counted
Section			
1. *Elatae* KÜKENTH.	*C. laevigata* SM.	36	2
2. *Distantes* LANG.	*C. hostiana* DC.	28	8
	C. punctata GAUD.	34	2
	C. distans L.	37	4
	C. binervis SM.	37	3
3. *Extensae* FR.	*C. flava* L.	30	7
	C. extensa GOOD.	30	3
	C. lepidocarpa TAUSCH.	34	50
	C. mairii COSS et GERM.	34	1
	C. demissa HORNEM.	35	49
	C. serotina MÉRAT	35	28
	C. scandinavica E. W. DAVIES	35	11
4. *Longirostres* KÜKENTH.	*C. sylvatica* HUDS.	29	3
5. *Rhomboidales* KÜKENTH.	*C. depauperata* GOOD.	22	1
6. *Gracillimae* CAREY	*C. strigosa* HUDS.	33	2
7. *Pachystylae* KÜKENTH.	*C. pallescens* L.	32	4
8. *Paniceae* TUCKERM.	*C. panicea* L.	16	5
9. *Trachychlaenae* DREJ.	*C. flacca* SCHREB.	38	7
10. *Hirtae* TUCKERM.	*C. hirta* L.	56	3
11. *Montanae* FR.	*C. pilulifera* L.	9	21
	C. ericetorum POLL.	15	2
	C. montana L.	19	3
	C. caryophyllea LATOUR.	32	2
		33	1
		34	2
12. *Digitatae* FR.	*C. humilis* LEYSS.	18	2
	C. digitata L.	24	1
		25	1
		26	2
	C. pediformis C. A. MEY.	35	1
13. *Atratae* KÜNTH.	*C. atrata* L.	27	2
14. *Fuliginosae* TUCKERM.	*C. frigida* ALL.	28	1
15. *Acutae* FR.	*C. bigelowii* TORR.	34	?
	C. elata ALL.	37	2
	C. aquatilis WAHLENB.	38	1
	C. caespitosa L.	40	1
	C. nigra (L.) REICHARD	42	2
16. *Bicolores* TUCKERM.	*C. bicolor* ALL.	25	1
Subgenus II. Vignea			
17. *Arenariae* KÜNTH.	*C. distacha* HUDS.	31	2
18. *Mühlenbergianae* TUCKERM.	*C. divulsa* STOKES	29	2
19. *Elongatae* KÜNTH.	*C. remota* L.	31	3

	Species	Haploid chromosome number	Number of plants counted
20. *Canescentes* FR.	*C. lachenalii* SCHKUHR.	29	1
21. *Ovales* KÜNTH.	C. ovalis GOOD.	32	2
Subgenus III. Primocarex			
22. *Pulicares* TUCKERM.	*C. peregrina* LINK.	31	1
23. *Dioicae* TUCKERM.	*C. dioica* L.	26	3

TABLE 2. *Chromosome numbers of some other genera in the Cyperaceae.*

Genus	Species	Haploid chromosome number	Number of plants counted from diff. localities
1. *Eriophorum*	*E. angustifolium* HONCK.	29	2
2. *Eleocharis*	*E. pauciflora* LINK.	10	2
	E. multicaulis (SM.) SM.	10	2
	E. palustris (L.) (ROEM.) SCHULT.	20	1
3. *Isolepis*	*I. setacea* (L.) R. BR.	13	2
4. *Schoenus*	*S. nigricans* L.	22	2

have three features, which are most unusual, if not unique, in the plant kingdom. These are the disintegration or abortion of three of the potential microspores from each tetrad (Plate III, Figs. 28—30); the post-reductional meiosis, in which the first meiotic division is considered in the main an equational division, while the second is reductional; and the long aneuploid series, the haploid numbers ranging from $n=6$ to $n=56$, and every number from 12 to 43 being represented by one or more species.

The pioneer in the study of this group was JUEL (1900), and he like STOUT (1913) investigated principally the peculiar method of pollen development. HEILBORN (1918, 1922, 1924, 1928, 1932, 1934, 1936, 1937, and 1939) has however done more to elucidate the cytology of this genus than any other worker, and was the first to count the chromosomes and study their morphology. He realized at an early stage that the numbers were quite irregular, and could not be arranged as a series of multiples; in fact, polyploidy of a normal type was absent.

He concludes from his observations that the lack of allopolyploidy may be related to the unique type of pollen development, for the inherent tendency of degeneration of three of the four nuclei in each tetrad prevents the formation of unreduced pollen dyads in any interspecific hybrid and therefore polyploidy occasioned by unreduced

gametes is completely lacking. However, he favoured the view that autopolyploidy may have played a major rôle in the early involution of the group, and shows that the statistical arrangement of the chromosome numbers in a graph pointed to 7 as the original basic number in the genus, as the most numerous multiple numbers recorded are 28, 42, and 56. Thus, he suggests that the most important processes producing aneuploidy in the *Cyperaceae* are autopolyloidy causing meiotic instability, and structural changes of the chromosomes such as fragmentation, failure of pairing giving rise to polysomics, and possibly to a lesser extent hybridizing.

TANAKA (1937, 1938, 1939) in Japan was the next worker to investigate the cytology of the *Cyperaceae* and his research was on species growing in the vicinity of Tokyo. His early papers were confined to some of the irregularities found at meiosis in different form of *Scirpus lacustris*, but in a later paper he gives a definite instance of autopolyploidy in *Carex sidérosticta*, $n=12, 24$.

WAHL in 1940 examined meiosis, and counted the chromosomes in a number of N. American species of *Carex*. From his results he agreed with HEILBORN that the aneuploid series was secondarily derived from an euploid one with different basic numbers, and that 7 was the most frequently represented.

He found in several cases different chromosome numbers or conditions, in the form of multivalents in different individuals of the same species, and assumed that this indicated a fairly high degree of polyploidy since the unbalance was not lethal to the plant. His study of hybrids was also instructive, and multivalents and univalents were of frequent occurrence. However, his most interesting observations were the absence of laggards at anaphase I, while these and bridge formations were seen at anaphase II. From this he concluded that in the genus *Carex* each chromosome splits longitudinally during the first meiotic division and therefore, apart from the possibility that chiasmata may have occurred, this division is equational, while the second division is reductional. Evidence supporting this postulate was that he always found the same number of chromosomes at metaphase I and II, while different and quite irregular numbers were frequently found in the microspore nucleus of a hybrid plant, or an individual with multivalents.

Finally, the most recent work on a member of the *Cyperaceae* has been carried out by HÅKANSSON (1954). He studied meiosis and pollen mitosis in x-rayed and untreated spikelets of *Eleocharis palustris*, and

suggested that the appearance and behaviour of the chromosomes indicated that they have a diffuse centromere.

Thus, with these interesting observations and conclusions as a background, it seemed of interest to examine as many different species as possible together with any natural and artificial hybrids.

III. MATERIAL AND METHODS

Any species that were obtainable from known localities were used in this survey of the genus *Carex*. Thus the plants examined are rather miscellaneous, and include species from twenty-two sections of three of the subgenera, collected in Great Britain, Spain, France, Italy, Switzerland, Scandinavia, and Madeira. Meiosis has also been examined in *Eriophorum*, *Eleocharis*, *Isolepis*, and *Schoenus* (Plate III, Figs. 31—36).

The cytological technique employed throughout these investigations is a modification of the aceto-carmine squash method by the addition of a drop of 4 % iron alum, after fixing the male spikelets in 1 : 3 acetic : alcohol. The preparations have been kept as permanent records and the photomicrographs preserved with an herbarium specimen of the same plant.

The *Carex* chromosome numbers recorded are listed in Table 1, and the species are arranged in order of ascending chromosome numbers in sections under their respective sub-genera. The classification followed is based on that given in »Flora of the British Isles» by CLAPHAM, TUTIN, and WARBURG, with the inclusion of non-British European species.

IV. RECENT CYTOLOGICAL OBSERVATIONS IN THE GENUS CAREX

1. The nature of the centromere

The chromosomes of the species of *Carex* which have been examined are very small, and appear to have no localized centromere. Certainly no centromere constriction of the usual type has been located in any of the six hundred preparations made and examined (Plates I and II, Figs. 1—2, 5—26). There is considerable evidence to support this postulate. The chromosomes lie very flat in the equatorial plane during metaphase I, while at anaphase I, as the daughter chromosomes separate, they are at first completely parallel to one another (Plates I and II,

Figs. 1—2, 9—11, 16, 17), but in later stages have their terminal regions leading in the poleward direction, and seem to be concave. Thus, the appearance is quite unlike that seen at meiosis of a species with chromosomes with a localized centromere, which during anaphase I, move with this region leading, drawing the chromosomes which appear curved and never equidistant along their length to opposite poles.

The recent work on the cytology of the *Juncaceae* by DE CASTRO (1950) and DE CASTRO and NORONHA-WAGNER (1952) in Portugal produces even more convincing evidence. These workers have demonstrated that the chromosomes of *Luzula* have no localized constriction, and as can be seen in Plate I, Figs. 3—4, there is a very striking similarity between them and those of *Carex* (Plate I, Figs. 1—2). Likewise DE CASTRO describes a similar sequence of events at meiosis in *Luzula*; and as in *Carex* the bivalents place themselves at right angles to the equatorial plate at metaphase I, while at anaphase I, the four chromatids of each bivalent are pulled to the poles by opposed centromeric forces, which seem to be located at the two ends. In this way they are stretched and eventually forced to break in the middle.

The order of the meiotic divisions and behaviour of the bivalents at anaphase I must now be briefly discussed, as it is generally accepted that the inversion of these two divisions can only take place when there is a diffuse centromere (DE CASTRO, 1950). These phenomena are found associated in the *Coccidaea* and *Aphidae* (RIS, 1942) and in the *Juncaceae* (DE CASTRO, 1950 and MALHEIRAS, DE CASTRO and CAMARA, 1947).

WAHL (1940) corroborating the observations of HEILBORN (1928), conclusively proved that apart from any chiasmata that might have occurred, the first meiotic division in *Carex* is equational and the second reductional for all the chromosomes. The absence of lagging chromosomes at anaphase I in the hybrids of the *C. flava* aggregate (DAVIES, 1955) supports the conclusions of WAHL.

A peculiar sticky matrix, which gives the chromosomes an irregular outline and causes them to agglutinate together at their ends, forming a network, has been frequently noticed in species which lack a localized centromere, and is to be seen at the first and second division of meiosis in several *Carex* species, for example, *C. flacca* (Plate II, Figs. 19—21) and *C. hostiana* (Plate I, Figs. 15). It is even more pronounced in *C. pilulifera* after treatment with x-rays (Plate I, Figs. 5—6). This chromosome stickiness was first observed in members of the *Coccideae* and *Aphideae* by RIS, and later found in *Luzula purpurea* LINK by DE CASTRO.

The spindle in *Carex* as in *Luzula* is broad and at metaphase I

(Plate II, Fig. 27), several spindle fibres being attached to each chromosome, while at anaphace I the spindle becomes more elongated. Thus, all the evidence points to the conclusion that *Carex*, like *Luzula* and certain insects, has chromosomes without a localized centromere.

The theory that chromosomes of this type can break and the fragments remain viable as separate entities, was finally confirmed by DE CASTRO, CAMARA and MALHEIRAS (1948), when they shattered the chromosomes of *Luzula purpurea* by means of x-rays. They found that the resulting fragments divided longitudinally at metaphase I, and continued life as distinct and viable entities. Thus, as in the hybrid *Carices* examined, the anaphasic movements were normal, and no lagging chromosomes were detected at the first division.

It is now clear that the behaviour of the *Carex* chromosomes after a similar treatment with x-rays would give conclusive evidence about the nature of the centromere in this genus. This work is in progress, but the results will be described in a later paper.

2. Chromosome number and morphology

Even this preliminary and rather limited survey of the genus shows that chromosome shape and size varies considerably in the different sections. Thus, all the hairy-fruited species included in sections *Montanae* (*C. pilulifera*, *C. ericetorum*, *C. montana*, and *C. caryophyllea*), and *Digitatae* (*C. humilis*, *C. digitata*, and *C. pediformis*), have few relatively large, square bivalents (Plate I, Figs. 1—2, 7—11), while in contrast those in section *Acutae* (*C. elata*, *C. aquatilis*, *C. caespitosa* and *C. nigra*), are very small, rectangular and much more numerous (Plate I, Figs. 12—13). The two closely related sections *Distantes* (*C. hostiana*, *C. punctata*, *C. distans*, and *C. binervis*) (Plate I, Fig. 14) and *Extensae* (*C. flava*, *C. extensa*, *C. lepidocarpa*, *C. mairii*, *C. demissa*, *C. serotina*, and *C. scandinavica* (Plate II, Figs. 16—18), as might be expected, have very similar chromosomes and it is difficult to distinguish the species of these groups on chromosome morphology alone. However, the chromosomes of the species in these sections are quite distinct from those of the *Acutae*, *Montanae*, and *Digitatae*, and at metaphase I, the bivalents are medium sized, slightly rounded, but very regular and uniform.

So few species in the other sections have been examined cytologically that it is impossible to make any generalizations, but it is clear that chromosome morphology is a useful taxonomic character, and supports the arrangement of the species in the sections based on morphological criteria.

On the whole, the species included in the same section seem to have near if not consecutive chromosome numbers, which give rise to a short and independent aneuploid series. Thus, the haploid numbers in *Distantes* range from 28 to 37, in *Extensae* from 30 to 35, and in *Acutae* from 34 to 42 (Table 1).

These short aneuploid series found in closely allied species with chromosomes of similar morphology lend further support to the view that the genus *Carex* has a diffuse centromere. The series have almost certainly arisen in the first instance by chromosome breakage, agmatoploidy, and hence the species evolved through small and gradual changes in the course of time. It would therefore seem likely that the lower-numbered species have given arise to the higher numbered, and consequently the series is ascending. However, the fact that the aneuploid series of numbers have arisen by fragmentation, explains why the species with the higher chromosome numbers are always found to have increasingly smaller chromosomes. The original postulate of HEILBORN that this was due to the limitation of the capacity of the cells to produce chromatin can now be disregarded.

Finally, variation in chromosome size within a species must be briefly mentioned, for although the chromosomes of some species, for example, those included in the section *Distantes*, *Acutae* and *Extensae* are remarkably uniform in length, and similar in their morphology, certain species have chromosomes showing a range of size and shape. HEILBORN in 1924 described three size groups amongst the chromosomes of *C. pilulifera,* which he designated »A» or long, »B» or medium and »C» or short chromosomes. From this he concluded that the »B» and »C» types had arisen from the »A» form by transverse fragmentation as their combined lengths seemed about equal to one long chromosome.

It is found that plants of the same species from different localities always have the same complement of long, medium and short chromosomes. The other species included in *Montanae* show similar size differences; *C. montana*, $n=19$, always has one pair of large chromosomes at meiosis, and medium and short bivalents also represented (Plate I, Fig. 8), while *C. ericetorum*, $n=15$, shows at least two sizes of bivalents (Plate I, Fig. 7). However, the species which appear to have the greatest range of chromosome size (within a single cell) are *C. paniceа*, $n=16$, in the section *Paniceae* (Plate II, Fig. 22), and *C. flacca*, $n=38$, in section *Trachychlaenae* (Plate II, Figs. 19—21).

3. Variation in chromosome number within a species

Another rather curious and unusual phenomenon noticed during the course of this investigation, was that although in the majority of species the chromosome number was very constant, in others variation in number and size was frequently found. Thus, three consecutive haploid numbers have been recorded in *C. digitata* ($n=24$, 25, 26) and four in *C. caryophyllea* (=31, 32, 33, 34) (Table 1).

Two of the plants of *C. digitata* examined cytologically came from Great Britain, while the third was collected in Skåne in southern Sweden. It is saticfactory to find that the latter specimen had the haploid number of $n=26$ and therefore agrees with HEILBORN's count, which was almost certainly made on Swedish material. However, the British *C. digitata* has different chromosome numbers, and as can be seen in Plate I, Fig. 11 in the Derbyshire plant, $n=24$, while in a specimen collected at Roudsea in Lancashire, $n=25$. This is interesting, as the haploid chromosome number of the very closely allied species *C. ornithopoda* counted by HEILBORN in 1924 is $n=23$. Thus, a short aneuploid series of chromosome numbers exists within these two related species. A rather similar situation was found in *C. caryophyllea* only here better preparations at meiosis have made analysis of the situation more possible. All the chromosome counts recorded have been made on British material collected from the limestone districts of Derbyshire and Yorkshire (Table 1). HEILBORN also examined this species, and records the haploid number $n=31$ from a Swedish specimen of *C. caryophyllea*. Thus, within this single species an aneuploid series with the consecutive numbers $n=31$, 32, 33, and 34, apparently exists.

The metaphase plates from which some of these counts were made are shown in Plate I, Figs. 9—10. In the specimen from Cressbrookdale in Derbyshire, 34 distinct bivalents can be counted, but although these are uniform in their general morphology, several of them seem slightly smaller than the rest (Plate I, Fig. 9). Meiosis in the other two plants is not quite so normal, and in some respects resembles the situation described by TANAKA (1937) in *Scirpus lacustris*.

The specimen collected on Ingleborough in Yorkshire with $n=33$, has 32 bivalents and one larger conglomerate structure, while the plant from Markland Crips in Derbyshire with $n=32$, has only 30 bivalents but two larger multivalents (Plate I, Fig. 10).

Consequently, at first, these plants were thought to be hybrids. However, on further investigation this was found not to be so, and analysis revealed that in each case the conglomerate structures consisted of two

smaller bivalents which due to some affinity or stickiness, remain attached, and appeared as one entity. Thus, it is assumed that the forms with the higher chromosome numbers have arisen from those with fewer chromosome by fragmentation, or those with apparently lower numbers have two or more chromosomes adhering together. These differences of chromosome number within a morphological species are now very much more easily explained in the light of the suggestion that the chromosomes of members of the *Cyperaceae* have a diffuse centromere. Conversely, these observations are evidence in support of the postulate, for the extra chromosomes in some forms of *C. caryophyllea* have probably arisen by agmatoploidy, and the fragments remain viable. Thus as »Position effect» can almost certainly be disregarded, the inheritable material in each cell has remained unchanged.

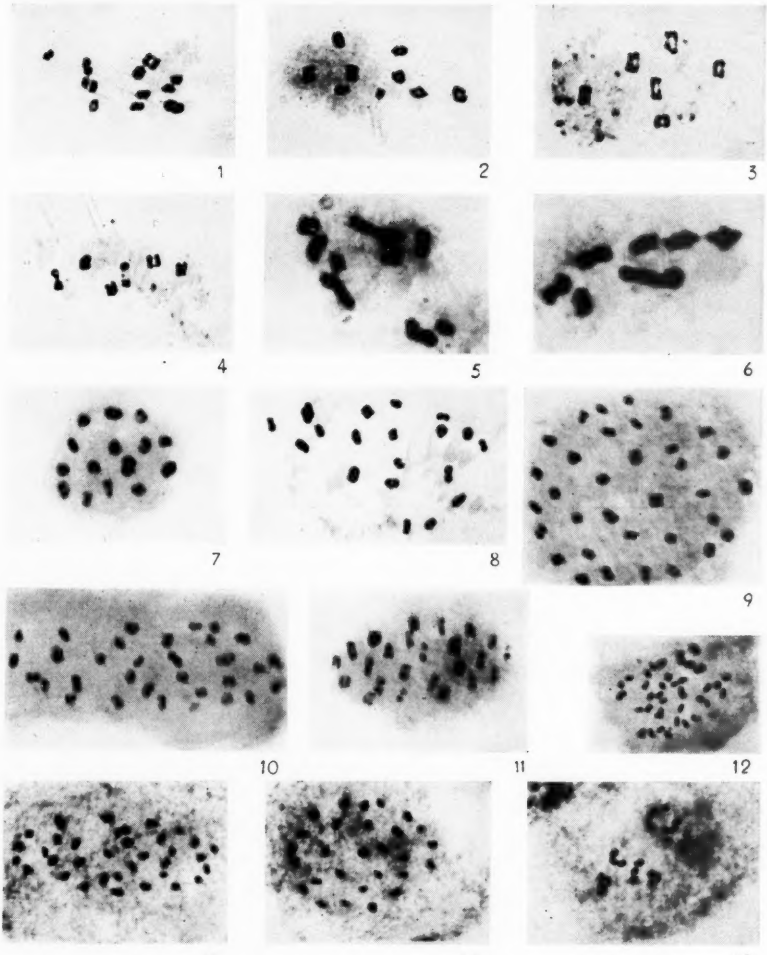
It seems likely that a cytological and taxonomic survey of *C. caryophyllea* from all over its geographical range would prove interesting. The variation in chromosome number often seems to have no influence on the external morphology, but the specimens from Cressbrookdale and Markland Crips in Derbyshire, with $n=32$ and 34, respectively, differ considerably from one another, the former plant being considerably larger and more robust than most forms of this species.

4. C. flacca SCHREB.

Meiosis in *C. flacca* (n=38) (Plate II, Figs. 19—21) must now be briefly discussed, for this species has chromosomes of very different sizes and some of the bivalents often appear joined by a narrow network of chromosome connections (Plate II, Figs. 19—21), which form a type of matrix. On further investigation the large chromosomes were found to consist of multivalents and the fragments of univalents as described by HEILBORN. The chromosome complement at meiosis was found to be the same in every specimen of *C. flacca* examined, when the multivalents had been carefully analysed and the number of chromosomes composing them ascertained. The number of multivalents and univalents, and amount of chromosome stickiness seemed to vary a great deal, and on a single preparation several superficially different and rather abnormal meiotic divisions were frequently found.

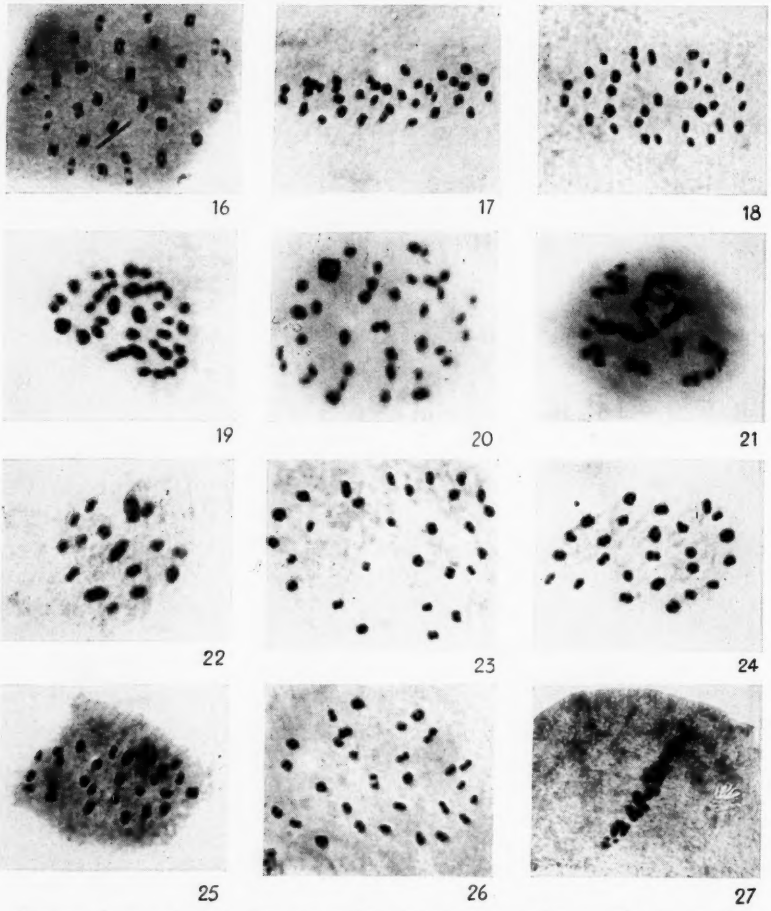
In many ways these metaphases (Plate II, Figs. 19—21) in *C. flacca* resemble those in *C. caryophyllea*, except that there was greater variation in the size of the multivalents and bivalents, and a more pronounced tendency for the chromosomes to adhere to one another. Meiosis in this species has more the appearance of that of a hybrid, if

Plate I.



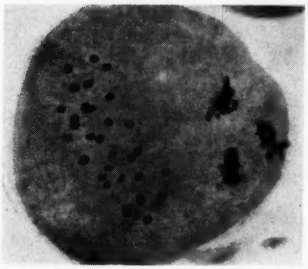
All figures show meiosis in the pollen mother cells (p.m.c.) Photographs 1—2, and 7—14, ×2400, 3—4, ×4000, 5—6, ×4800, 15, ×3600. — Figs. 1—2. *Carex pilulifera*, Bingley moor, Yorks., $n=9$. Early anaphase I showing daughter chromosomes separating parallel to one another, with their terminal regions leading in the poleward direction. — Figs. 3—4. *Luzula nemorosa*. $n=6$, showing metaphase I—anaphase I with bivalents at right angles to the metaphase plate separating parallel to one another. (After NORONHA-WAGNER and DE CASTRO, 1952.) — Figs. 5—6. *C. pilulifera*, Leicestershire, $n=9$, showing fragmentation and chromosome stickiness at metaphase I after treatment with 1000 r. — Fig. 7. *C. ericetorum*, Markland Crips, Derbyshire, $n=15$. Metaphase I showing two sizes of bivalents. — Fig. 8. *C. montana*, Anston Stones Wood, $n=19$. Metaphase I showing one pair of large chromosomes, medium and short bivalents. — Fig. 9. *C. caryophyllea*, Cressbrookdale, Derbyshire, $n=34$. Metaphase I. — Fig. 10. *C. caryophyllea*, Markland Crips, Derbyshire, $n=32$. Metaphase I with 30 bivalents and two larger multivalents. — Fig. 11. *C. digitata*, Markland Crips, Derbyshire, $n=24$. Early anaphase I showing daughter chromosomes moving to opposite poles, parallel to one another. — Fig. 12. *C. caespitosa*, Denmark, $n=40$. Metaphase I showing very small rectangular chromosomes. — Fig. 13. *C. bigelowii*, Ben Lawers, Mid. Perth., $n=34$. Metaphase I. — Fig. 14. *C. hostiana*, Ben Lawers, Mid. Perth., $n=28$. Metaphase I. — Fig. 15. *C. hostiana*, Ben Lawers, Mid. Perth., $n=28$. Metaphase II showing chromosomes sticking together forming a network.

Plate II.



Figures show meiosis in the p.m.c. All photographs ×2400. — Fig. 16. *C. flava*, Malham Moss, Yorks., $n=30$. Early anaphase I showing daughter chromosomes separating parallel to one another with their terminal regions banding in the poleward direction. — Fig. 17. *C. demissa*, Loch Tummel, Mid. Perth., $n=35$. Late metaphase I—early anaphase I. — Fig. 18. *C. mairii*, Lumbreras, Spain, $n=34$. Metaphase I showing secondary association. — Figs. 19—21. *C. flacca* from Leicestershire, Yorkshire, and Somerset, respectively, $n=38$. Metaphase I showing different sizes of bivalents joined by a narrow network of chromosome connections. — Fig. 22. *C. panicea*, Shapwick, Somerset, $n=16$. Metaphase I showing different sizes of bivalents. — Fig. 23. *C. frigida*, Albula Pass, Switzerland, $n=28$. Metaphase I. — Fig. 24. *C. bicolor*, Preda, Switzerland, $n=25$. Metaphase I. — Fig. 25. *C. sylvatica*, Abbots Leigh, Somerset, $n=29$. Early anaphase I. — Fig. 26. *C. lachenalii*, Abisko, Sweden, $n=29$. Late metaphase I—early anaphase I. — Fig. 27. *C. bigelowii*, Ben Lawers, Mid. Perth. Showing broad, truncate spindle in metaphase I.

Plate III



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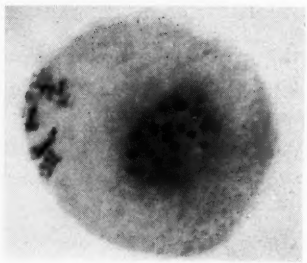

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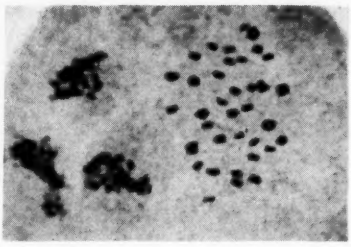

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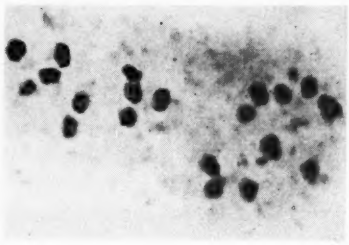

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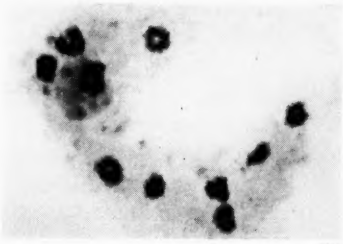

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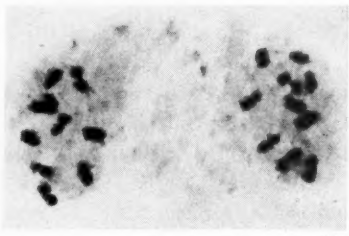

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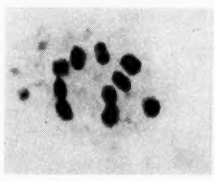

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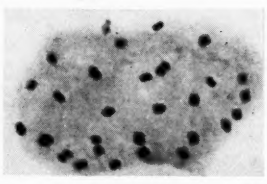

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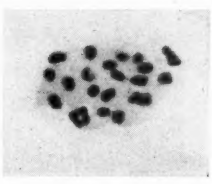

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All photographs ×2400. — Fig. 28. *C. flava*, Rondsea Wood, Lancs., $n=30$. Pollen grain mitosis, and abortion of three of potential microspores in tetrad. — Fig. 29. *C. digitata*, Markland Crips, Derbyshire, $n=24$. Pollen grain mitosis, and three aborting nuclei. — Fig. 30. *C. pediformis*, Sweden, $n=35$. Pollen grain mitosis, and three aborting nuclei. — Fig. 31. *Eleocharis palustris*, Holmsey Bog, Hants, $n=20$. Metaphase I in p.m.c. — Fig. 32. *E. multicaulis*, Derby Fen, Norfolk, $n=10$. Diakinesis in p.m.c., showing the nucleolus and some spiral structure. — Fig. 33. *E. multicaulis*, Derby Fen, Norfolk, $n=10$ Late anaphase I in p.m.c. — Fig. 34. *E. multicaulis*, Derby Fen, Norfolk, $n=10$. Late metaphase in p.m.c. — Fig. 35. *Eriophorum angustifolium*, Teesdale, Durham, $n=29$. Late metaphase I—early anaphase I in p.m.c., showing daughter chromosomes separating parallel to one another with their terminal regions leading in the poleward direction as in *Carex*. — Fig. 36. *Schoenus nigricans*, Wittering Marsh, Northants., $n=22$. Metaphase I in the p.m.c., with one large chromosome pair or multivalent.

not an autopolyploid, and its chromosome morphology is quite unlike any other species of *Carex* so far examined. Likewise, the almost complete sterility of *C. flacca* with a germination of less than 5 % produces further evidence in favour of the unstable nature of this plant; but its origin is difficult to explain, as it seems to be isolated, with no close allies in Northern Europe.

It is still not clear whether autopolyploidy, hybridity, or merely chromosome stickiness is the most important cause of the peculiar and varied appearance of the meiotic chromosomes in *C. flacca*. The sterility of the species certainly supports one of the first two hypotheses, and this chromosome stickiness may well be associated with one of these, due to the unstable state of the species. Certainly, the curious affinity between some of the bivalents is so similar to the network of associated chromosomes noticed at meiosis in *Luzula purpurea* (DE CASTRO, CAMARA, MALHEIROS, 1947), that it is difficult to disregard this analogy entirely. It is hoped that a more intensive study of this species, including an examination of the behaviour of the chromosomes at anaphase I and II during the pollen grain mitosis, will help to elucidate the problem and throw some light on the origin and nature of *C. flacca*.

5. Polyploidy

Polyploidy appears to be unimportant in the evolution of the genus *Carex*, though one example has been found during the course of this investigation. *C. humilis* collected in the Avon Gorge, Bristol, has the haploid number $n=18$, and is almost certainly a diploid, while plants examined by TANAKA (1942) were tetraploid with $n=36$. Since this difference was detected, specimens of *C. humilis* have been obtained from Switzerland and Germany. These plants differ from British ones in being much more robust, with considerably larger and more trailing leaves. These plants have not yet produced any suitable fertile spikes, so they have not been examined cytologically.

It is not known whether this is a case of autopolyploidy or allopolyploidy, as TANAKA does not mention the behaviour of the chromosomes at meiosis. However, it seems likely that this is an isolated example of the former, and *C. humilis*, like *C. siderosticta*, thus consists of two polyploid races.

6. Secondary association

This phenomenon has not been noticed to any great extent in the genus *Carex*, although some bivalents in a few species (Plate II,

Figs. 18—24) do tend to remain in pairs or at least fairly closely associated on the metaphase plate. This is most often found in species with chromosomes of different size groups, which HEILBORN suggests may be merely the expression of the tendency for chromosomes of a similar size to become associated due to the sorting processes coincident with cell division. Chromosome stickiness which produces a network in certain species, seems a more probable explanation, especially as it is more often found in plants that possess chromosomes of different sizes.

These rare occurrences of secondary association (which in any case may not be due to chromosome homology) seem a further indication that autopolyploidy is exceedingly rare in most species of *Carex*, and consequently plays a very small part in the evolution of the genus.

7. The chromosomes of some other members of the Cyperaceae and their relationships with Carex

A comparison of the chromosomes of *Eriophorum*, *Schoenus*, *Isolepis*, and *Eleocharis* with those of *Carex* is interesting, and clearly indicates a close cytological similarity between the former two and *Carex*, while in contrast those of *Isolepis* and *Eleocharis* seem to be rather different.

The chromosomes of *Eriophorum angustifolium*, $n=29$, Plate III, Fig. 35) and *Schoenus nigricans*, $n=22$ (Plate III, Fig. 36) are almost indistinguishable from the chromosomes of species of *Carex*, while those of *Isolepis setaceus*, $n=13$, and *Eleocharis multicaulus*, $n=10$, and *E. uniglumis*, $n=20$ (Plate III, Figs. 31—34) are very different. Even when it is taken into consideration that the chromosomes of *Eleocharis palustris* have been photographed at diakinesis (Plate III, Fig. 32) they are considerably larger than most *Carex* chromosomes, and their spiral structure is visible, a detail that has never really been seen in *Carex*.

Thus, the cytology supports the morphological evidence that the relationships of *Carex* with *Scirpus*, *Eleocharis*, and *Isolepis* are rather distant. Before drawing any conclusions from these very limited observations, it will be necessary to examine some of the intermediate genera such as *Blysmus*, *Kobresia*, and *Trichophorum caespitosus*, for these show some characteristics of both.

V. CONCLUSIONS

It seems that autopolyploidy may have played a minor part in the early evolution of this genus, and occasional instances are still to be

found. However, aneuploidy is undoubtedly the major process in speciation of *Carex*, and the diffuse nature of the centromere almost certainly account for the long aneuploid series that is present in the *Cyperaceae*. The fact that the chromosomes in this family, as in *Juncaceae*, can break and the fragments remain viable and continue to function as separate entities, explains both the series of quite irregular numbers, and the decrease in chromosome size with increase in number.

It is clear that the aneuploid series in the *Cyperaceae* is quite distinct from the types recognized by STEBBINS (1950). He distinguishes ascending and descending series, produced by means of unequal translocations which give rise to polysomics and eventually an aneuploid series. The genus *Crepis* studied in great detail by BABCOCK (1937) exemplifies this type of aneuploidy with an ascending series.

It is still uncertain what causes the chromosome fragmentation, but it is possible that breakage might occur spontaneously, or under the influence of cosmic rays or other external forces, so producing an aneuploid series in this genus, in the same way that polyploidy has given rise to new and vigorous species in other genera. Aneuploidy thus seems to be an ascending process in the genus *Carex*, and the more recent species the more numerous and smaller chromosomes. Like polyploidy, aneuploidy is an evolutionary process producing variation in plants, and hence new and adaptable species that can colonize changing habitats, and new environments which may arise.

In contrast to polyploidy, aneuploidy does not appear to increase with latitude. HASKELL (1952) has shown that in the British Isles there is no relationship between distribution and chromosome number in *Carex* species, ones with low (*C. pilulifera*, $n=9$) and high (*C. hirta*, $n=56$) chromosome numbers both having a wide distribution.

SUMMARY

This paper is confined to a detailed cytological survey of the genus *Carex*.

(1) A brief survey of previous cytological work in this genus is given.

(2) The diffuse nature of the centromere, the behaviour of the chromosomes at meiosis, and the phenomenon of chromosome stickiness are discussed.

(3) The variation in chromosome size and number in different sections of the genus is mentioned, and the use of cytology as a taxonomic character is suggested.

(4) Several consecutive chromosome numbers have been shown to occur within *C. caryophyllea* and *C. digitata*, and the significance of these short aneuploid series within a species is discussed.

(5) The abnormalities such as multivalents and chromosome stickiness found at meiosis in *C. flacca* are described and some reasons for these irregularities are put forward.

(6) One example of polyploidy in *C. humilis* is reported, and the rarity of this and secondary association in the genus is discussed.

(7) The chromosomes of some other genera in the *Cyperaceae*, namely *Eriophorum*, *Schoenus*, *Eleocharis*, and *Isolepis*, are compared with those of *Carex*.

(8) The conclusions reached are as follows:

Aneuploidy is the major process in the evolution of *Carex*, and the diffuse nature of the centromere accounts for the long aneuploid series present in this genus. The chromosomes of the *Cyperaceae*, like the *Juncaceae*, break and the fragments remain viable and function as separate entities. Thus, the series is ascending, and like polyploidy, aneuploidy in the form of agmatoploidy, produces variation in plants and speciation.

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SELF-PERPETUATING RING CHROMOSOMES IN TWO HUMAN TUMOURS

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OBSERVATIONS

EXPLORATIVE studies into the chromosomes of some twenty human effusion tumours revealed two tumours with self-perpetuating ring chromosomes in their stemline. The histo-pathology and general chromosome cytology of these tumours have been reported by ISING and LEVAN (1956). The present paper deals with the structure and behaviour of the ring chromosomes.

As reported in the paper mentioned the two tumours were one pleural exsudate from a lung carcinoma (l. t.) and one ascites of a stomach tumour (s. t.). Both tumours had a hypertriploid stemline with modes at 75 and 82 for the l. t. and s. t., respectively. The l. t. had one ring chromosome in all analyzable cells. This ring persisted in the stemline through two samplings with an interval of two weeks; the s. t. often had two rings per cell. The ring of the l. t. was larger, being the tenth longest chromosome of the idiogram, while the s. t. rings came as numbers 22 and 23 in their idiogram. The absolute chromosome length averaged 6 and 4 μ, respectively, for the l. t. and s. t. rings, the l. t. ring thus being about 1,5 times larger. In addition, the l. t. ring was more favourable for analysis so that most descriptions below are based on this ring.

The rings *in situ* at metaphase in the l. t. and s. t. are seen in Figs. 1 *a* and *b*, respectively. Several instances of individual rings at metaphase, as compared with other chromosomes of the same plates, among them the largest and smallest ones, are drawn in Fig. 2 *f*—*j* for the l. t. and Fig. 1 *g*—*i* for the s. t. It is possible to trace the ring structures back into early prophase, a few instances of prophase rings being pictured in Fig. 2 *a*—*e*. Like most, perhaps all, human chromosomes the rings have heterochromatic segments on both sides of the centromere. These segments often fuse into one knob (Fig. 2 *a*) but sometimes show four distinct parts (Fig. 2 *e*). During the transition from prophase to metaphase the rings, as well as the other chromosomes, grow more stainable and more contracted. A series of different contraction stages is given in

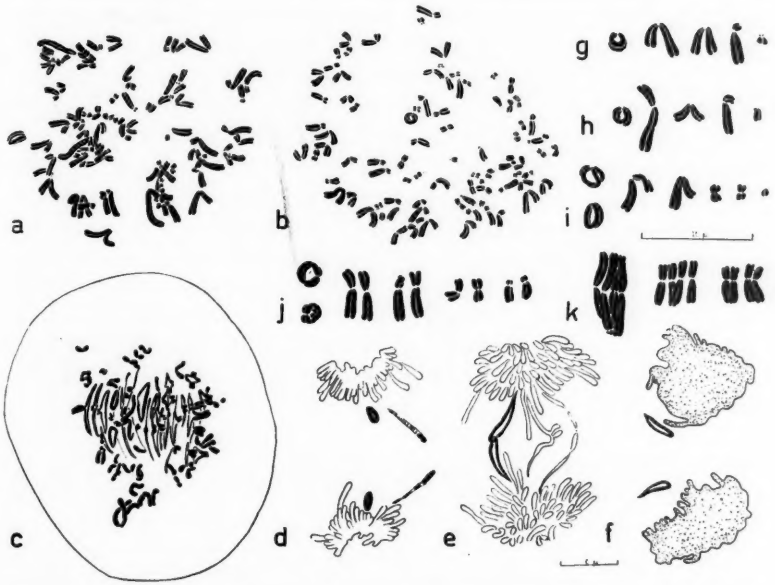

Fig. 1 *a*, *c*—*f*: the lung tumour; *b*, *g*—*k*: the stomach tumour; *a*, *b*: metaphases in polar view; *c*: anaphase in side view, chromosomes of d-type (see TJIO and LEVAN, 1954); *d*—*f*: late anaphase and telophase with free rings (*d*, *f*), and interlocked rings (*e*); *g*—*j*; condensed idiograms; *j*: endoreduplicated cell; *k*: instances of quadruplochromosomes. — *a*—*f*: ×850; *g*—*k*: ×1700.

Fig. 2 *k*. This variation according to mitotic stage renders difficult the comparison of rings from different cells. The size of the rings in the same tumour seems to be fairly constant. Sometimes, however, clearly deviating rings are observed, both smaller and larger ones. Disregarding the dicentrics, which are usually double-sized, very large monocentric rings were recorded. The rings of Fig. 2 *r*, for instance, had only one centromere, although it had the size of the ordinary dicentrics. As is well known, size variation due to deletion and duplication is a characteristic feature of rings.

The behaviour of rings at anaphase is especially interesting, and gives the background for their variability. It has been established that three main types of anaphase structures may occur: (1) two free, »disjunctional» anaphase rings, identical with the original ring; (2) one large dicentric ring, of double size as compared with the orginal ring; and (3) interlocked rings, each of the size of the original ring. In case

Fig. 2. The behaviour of the ring chromosome of the lung tumour; *a*—*e*: prophase; *f*—*k*, *q*—*x*: metaphase; *l*—*p*: anaphase; *n* is the same cell as in Fig. 4 *c*; *q*, *s*—*x*: endoreduplications. — ×2000.

(1) the anaphase separation meets with no difficulties, the ring being brought over unchanged to the daughter nuclei. In cases (2) and (3), however, a double bridge will be formed between the anaphase groups. Case (3) is difficult to distinguish from (2) at this stage, especially as a twist may occur in the double-sized ring of (2). The configurations (2) and (3) will lead to the elimination of the ring chromosome, to non-disjunctional distribution of the whole structure, or to the chromatids breaking before telophase, allowing the broken parts of the rings to be distributed to the telophase nuclei. In the last case deletions and duplications occur, unless the point of breakage is exactly halfway between the centromeres. Later on the broken ends will join inside each daughter nucleus, either the two ends of the same chromosome, or the sister-strands of the half-chromatids at each chromosome end. In the former case, (1), (2) or (3) will again appear at the next mitosis; in the latter case (2) will appear.

In our material all three cases of anaphase configurations were seen. Free anaphase rings are pictured in Fig. 1 *c*, *d* and in Fig. 2 *l*, *m*, *n*. It should be noted in passing that the cell of Fig. 1 *c* (details in Fig. 2 *n*) represents the interesting deviation of mitosis, called d-mitosis (TJIO and LEVAN, 1954), characterized by strongly pulled-out centromeric regions and a slowly progressing lapse of chromatid association. Large dicentric rings are seen in Fig. 2 *o*, and interlocked rings in Fig. 1 *e* and Fig. 2 *p*. The last case is naturally the most difficult one to identify; it was observed unequivocally only twice. Double bridges between anaphase groups and telophase nuclei were frequent, especially in the l. t. On one occasion 19 among 56 cells (34 %) were recorded with double bridges, most of which should represent dicentric anaphase rings.

As reported by ISING and LEVAN (*l. c.*), the present tumours are characterized by a frequent incidence of endo-reduplication, giving rise to diplochromosomes (Fig. 2 *x*) and sometimes even to quadruplochromosomes (Fig. 1 *k*). Specific attention was paid to the behaviour of the rings during endo-reduplication. This is an opportunity for studying two or more chromosome reproductions at one mitosis. We found the expected configurations of two free rings of normal size (Fig. 1 *j*, Fig. 2 *q*) about as equally frequent as one double-sized, dicentric ring (Fig. 2 *t*, *u*). In some cases one cell contained two free rings and one large dicentric ring (Fig. 2 *s*). Two interlocked rings were never seen with certainty. Fig. 2 *w* and *x* may represent such cases, but the course of their chromatids was not quite clear.

The remarkable case of four interlocked rings forming a chain

(Fig. 2 *v*) was found in a cell with quadruple chromosome number, the rest of the chromosomes being arranged in quartets. This structure is unparalleled in chromosome experience, as far as we know, and may have an interesting bearing on the mechanism of the mitotic variations of the rings, as discussed in the next section.

DISCUSSION

Ring-shaped chromosomes are formed from metacentric chromosomes by structural interchange between the two arms of one chromosome. Ring chromosomes have been produced experimentally in plants and insects by irradiation; they have also been found in spontaneous material in a few extremely rare cases. Thus, a varying number of rings occurred in the root meristem of one seedling obtained from a normal *Crepis tectorum* plant (NAVASHIN, 1930). Up to four rings were present in the roots of one progeny plant from six individuals of *Centaurea scabiosa*, collected in their natural habitat (FRÖST, 1948). In *Drosophila*, rings are found in progenies from attached X females, their origin being due to crossing-over within an inverted segment or to interchange between the two arms of the attached X (SCHULTZ and CATCHESIDE, 1937). The present cases seem to be the first ones on record of self-perpetuating rings in a mammalian tissue. They should be expected in cancer tissue as a result of the high incidence of spontaneous structural rearrangements, characteristic of cancer. Occasional ring chromosomes were observed by TJIO and LEVAN (1954) in mouse tumours.

The most far-reaching analysis of the behaviour of ring chromosomes and the effects of their mitotic variation has been made by MCCLINTOCK (1938, and elsewhere) in *Zea*. By producing rings with marker genes MCCLINTOCK managed to make the effects of changes within the rings visible macroscopically.

Two alternative hypotheses have been put forward to interpret the origin of double-sized dicentrics and interlocked rings. The one interpretation calculates with torsions of the division plane of the ring chromosome. If no torsions occur, free rings would result; an odd number of torsions give double-sized rings; an even number of torsions give interlocked rings. The other view assumes somatic crossing-over between the two chromatids of the ring. One chiasma would give rise to double-sized rings, while two non-compensating chiasmata would result in interlocked rings. While HUSTED (1936) from results in *Tradescantia* favours the former hypothesis, certain of MCCLINTOCK's data are more

compatible with the latter hypothesis. Thus, the continuous new-formation of interlocked or double-sized rings from disjunctional rings is more in agreement with chiasma formation, because according to the torsion hypothesis a ring, once disjunctional, would continue to form free rings. Likewise, evidence from maize meiosis (SCHWARTZ, 1953) indicates a frequent occurrence of sister-strand crossing over.

The chain of four interlocked rings observed in the present study (Fig. 2 *v*) belonged to a cell, in which all chromosomes had reproduced three times during the preceding interphase, forming quadruplochromosomes. Now it seems that mere triple reproduction of an ordinary ring would be unable to lead to such an end, no matter what torsions of the division plane are imagined. In our opinion crossing-over or some other kind of structural rearrangement involving breakage and reunion has to be assumed in order to explain the formation of this chain. One may hesitate between somatic crossing-over between pairs of chromatids and breakage and reunion within single chromatids. As a matter of fact, such a chain of four rings could originate by only two occurrences of the latter kind, the first one affecting a daughter strand of the first order, and the second one a daughter strand of the second order. By the first occurrence two interlocked rings would originate, by the second the closed circle of four rings, thus formed, would open up into a chain.

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SUMMARY

The ubiquitous occurrence of ring-shaped chromosomes in the stemline of two human tumours has provided the opportunity for a study of the structure and transformations of the rings during ordinary mitosis and endomitosis. The configuration of Fig. 2 *v*, *viz.* a chain of four interlocked rings, indicates that structural rearrangements between sister-strands are in action at the transformations of the ring.

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SEED DEVELOPMENT OF BRASSICA OLERACEA AND B. RAPA AFTER CERTAIN RECIPROCAL POLLINATIONS

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I. INTRODUCTION

HOWARD (1939) was the first to report poor seed quality after pollinations between diploids and their autotetraploids in *Cruciferae*. *Brassica oleracea* produced no triploids after 2X×4X pollinations, the seeds formed being shrivelled and empty; a few good seeds resulted in diploid or tetraploid seedlings. The reciprocal pollination, 4X×2X, was more successful, and quite a considerable number of seeds were formed. These were small, and their germination resulted in triploid seedlings. OLSSON and RUFELT (1948) reported similar experiences during their crossings with *Sinapis alba*; empty seeds after 2X×4X, small seeds giving triploids after 4X×2X. In *Raphanus sativus* the pollination 4X×2X was successful but all attempts to perform the cross with the diploid as mother plant failed (NISHIYAMA, 1948, 1952). Somewhat different results have, however, been obtained in *Brassica chinensis*, *B. pekinensis* and *B. rapa* (=*B. campestris*), three species which are closely related, being more of the nature of subspecies (*cf.* OLSSON, 1954, p. 415). NISHIYAMA and INAMORI (1953) obtained no triploids after 4X×2X, both the reciprocal pollinations resulting in poor seeds. This was also the result of the crosses between diploid and autotetraploid *Nasturtium officinale* (HOWARD, 1947).

The embryology of these interesting cases of seed failure has hitherto not been investigated. However, I have had the opportunity to study seed development after reciprocal pollinations between diploid and tetraploid plants of *Brassica oleracea* and of *B. rapa*. Development after interspecific pollinations between these species could also be investigated. The material was sent to me from Svalöf. The experiments had shown that no triploid *B. rapa* could be produced, whereas triploid *B. oleracea* appeared in 4X×2X crosses. In the interspecific crosses between these species only very rarely were hybrids produced. The chro-

mosome numbers of the two species are, in kale $2n=18$, in turnip $2n=20$.

The investigated material consisted of siliqua from flowers which had been emasculated and artificially pollinated after $1^1/_2$—2 days. Fixations had been made at various times after pollinations, usually after 5, 10, 15 hours and after 1, 3, 6, 10, 14, 21, and 28 days. The early collections were made in the hope of establishing differences of growth rates of pollen tubes after different pollinations but the conditions were unfavourable to such investigations. Most fixations were performed in 1954, but the description of the development after interspecific crosses is based on fixations made early in the summer of 1955. The collection of the investigation material has been supported by grants from the Swedish Agricultural Research Council.

II. NORMAL SEED DEVELOPMENT IN CRUCIFERAE

The ovule of Crucifers has two integuments and a rather long and thin nucellus. The enlarging young embryo sac destroys the apical part of the nucellus, and consequently the mature embryo sac is surrounded by the inner integument. It has two enlarged polar nuclei lying close together near the egg-apparatus, while the three antipodals are collapsed. Below the antipodals the long lower part of the nucellus persists; this is not united with the inner integument, and later the embryo sac grows down between nucellus and integument.

Seed development after fertilization may be briefly summarized according to descriptions in the literature and from my own experiences. Immediately after fertilization, growth of ovule and embryo sac is resumed. During further growth the amphitropous curvature of ovule and embryo sac becomes more pronounced; the embryo sac with endosperm nuclei then takes the form of a horseshoe, the two ends being in rather close vicinity (cf. Fig. 1). The still persisting part of the nucellus is dissolved, only a convex elevation being left above the chalaza. If fertilization fails the nucellus does not disappear. The sporophytic tissue of the ovules enlarges after fertilization, particularly the inner integument. Soon starch is stored in those parts of the inner integument which are nearest to the endosperm, while its outer layers under normal conditions remain free from starch. The inner integument is ephemeral. Later the outer integument also contains starch except the cells of its inner epidermis which forms the most important layer of the seed coat. The outer integument persists. In the basal part of the seed as well in

the hilum starch is also stored except in the regions of the nucellar base and chalaza, the tissue here often adopting a peculiar structure.

Endosperm formation follows the Nucleate type. It begins immediately after fertilization, the polar nuclei fusing and moving from the egg-apparatus. Fairly early on the formation of a basal endosperm begins on the convex elevation near the chalaza. This endosperm never becomes cellular. It has a dense cytoplasm and later becomes sharply delimited from the main endosperm (Fig. 1). Probably this basal endosperm has some nutritive function during seed development. Its nuclei may show a tendency to increasing size. There is a conical assemblage of endosperm surrounding the suspensor of the young embryo. This may be called apical endosperm, but it is less distinct than the basal endosperm; it later becomes cellular. Cell formation begins in the micropylar part of the endosperm, a tissue being formed which then grows centripetally towards the centre of the large central vacuole; it also grows in centrifugal directions encroaching upon the inner integument. The endosperm may be a considerable tissue but is always ephemeral. RIJVEN (1952) has established the presence of sugars, starch and amino-acids in the endosperm sap of *Capsella bursa-pastoris*. In the slides examined here starch generally seemed to be absent from the endosperm, which in this respect differed from the sporophytic tissue. Mature seeds are endospermless. However, sometimes the persistence of some endosperm layers has been reported (see, for instance, STOKES, 1955).

Embryo formation of Crucifers has often been closely investigated. It is initiated through a considerable elongation of the egg, which is then divided near its apical end. A second division completes the proembryo, which consists of a row of three cells. Longitudinal division of the apical cell initiates the forming of an embryo globe, while the long basal cell of the proembryo is repeatedly divided transversely, a suspensor of a row of cells being the result. RIJVIN (1952) has published an important study of *in vitro* growth of embryos of *Capsella bursa-pastoris*. He discerns eight stages of embryo development in the ovule. They are described here after WARDLAW (1955). I. Globular, the embryo forming a globe (18—50 μ in *Capsella*). II. Reversed trapezium (50—80 μ). III. Heart stage, the cotyledons appearing (80—150 μ). IV. Intermediate stage, growth of cotyledons (150—350 μ). V. »Torpedo», the cotyledons are flattened against each other and constitute half the length of the embryo (350—700 μ). VI. »Walking-stick», caused by the cotyledons having turned back in the top of the ovule (700—900 μ). VII. Upturned U, the one leg formed by the hypocotyle, the

other by the flattened cotyledons (900—1700 μ). VIII. Fullgrown embryo. From the heart stage onwards embryos may be cultivated *in vitro*, young embryos being green. In the morphogenetic development the heart stage is important »in that it marks the transition from the initial radial symmetry to the bilateral symmetry that becomes apparent with the formation of the cotyledons» (WARDLAW, *l. c.*, p. 309). The change of symmetry seems induced from factors outside the embryo, some *in vitro* embryos not losing their radial symmetry and developing as many as six cotyledons. The mature embryos of *Brassica* have long cotyledons, which are folded longitudinally.

III. BRASSICA RAPA

2X×*2X*. — Fertilized ovules were found 1 day after pollination, and contained a few endosperm nuclei (2—8). After 2 days the egg may be elongated but a proembryo was only observed after 3 days. At that time the nucellus had disappeared and some endosperm nuclei were assembled on the elevation above the chalaza. Thus, after the formation of the basal endosperm had been initiated, eight nuclei were now usually seen in the chalaza. The most common number of endosperm nuclei was 32 or 64 (in collections from 1953, 128). Some starch had appeared in the inner integument. Later the embryo sac had more or less the form of a horseshoe (»inverted U»). After 6 days there was a globe embryo. The globe may only count twenty cells but was generally larger with complete dermatogen and with 5—6 or 8 cells along its diameter. The endosperm was a single layer of free nuclei in the cytoplasm surrounding the central vacuole. There was now a distinct apical as well as basal endosperm (cf. Fig. 1), and more starch was appearing in the inner integument.

After 10 days the ovules had much increased in size, both integuments having much starch. The embryo had passed the heart stage and was now having small cotyledons. It mostly showed stage IV, approaching the form of a torpedo. It was still small compared with the voluminous endosperm, which had a very large central vacuole. Cells had often been formed in the apical endosperm and neighbouring parts of the endosperm. The main part of the endosperm was still nuclear but vacuoles had generally appeared between the nuclei as an initiation of cell formation. After 14 days the endosperm may have maximal development. It is cellular and occupies much of the central vacuole, but the latter is probably as a rule never completely filled. Very long endo-

sperm cells exist in certain parts of the seed, and elongate towards its centre. A more rapid growth of the embryo had now set in, the most advanced embryos showing cotyledons which were possibly bent near the apical ends, being at the outset of the »walking-stick» stage. The inner integument tissue had now diminished. After 21 days most of the sporophytic tissue as well as the endosperm had disappeared, a large embryo now filling the space within the testa. After 28 days the cotyledons had attained full size, the embryo filling the seed.

4X×4X. — Ovule and embryo sac are larger than in diploid turnip. A small number of ovules lack embryo sac owing to degeneration of the macrospores. Such ovules have normal integuments enclosing an intact nucellus with dead macrospores or a degenerated e.m.c. In diploids, sterile ovules are very rare. The antipodal cells were seemingly unusually long-lived, being distinct also in the mature embryo sac. A similar difference between the antipodals of a diploid and its autotetraploid was also observed in *Galeopsis pubescens* (HÅKANSSON, 1952).

The result of the artificial pollinations investigated indicate that the failure of fertilization is more frequent in tetraploid turnip. The rate of development after fertilization was more variable than in the diploid; it is interrupted in a number of ovules. However, young seeds, having a more rapid development, were similar to the seeds of the diploid. After 1 day two endosperm nuclei may be observed. After 3 days and 6 days, development may have proceeded as in the diploid. After 14 days, the endosperm is cellular forming a tissue around the central vacuole. Its peripheral cells are small, their divisions causing centrifugal growth of the endosperm. The embryo may be a »walking-stick»; more often it was a »torpedo». After 21 and 28 days the embryo often filled the seed. Certain seeds still contained some endosperm; the nuclei of old basal endosperms may be enlarged.

2X×4X. — The pollinations had, as a rule succeeded, though the number of fertilized ovules in the siliqua varied. The initial development of the seeds was normal. 1 day after pollination there could be two or four endosperm nuclei, and after 3 days a proembryo was sometimes formed. In the endosperm 32 nuclei were counted, four of them near the chalaza (compare 2X×2X). The siliqua, fixed 6 days after pollination, were unfortunately in a rather poor condition, only a few good ovules being found. An embryo globe counting 16 cells was observed. The endosperm was fairly normal, though the apical and basal endosperms were as a rule under-developed.

A slow development was evident after 10 days (Fig. 3). The young

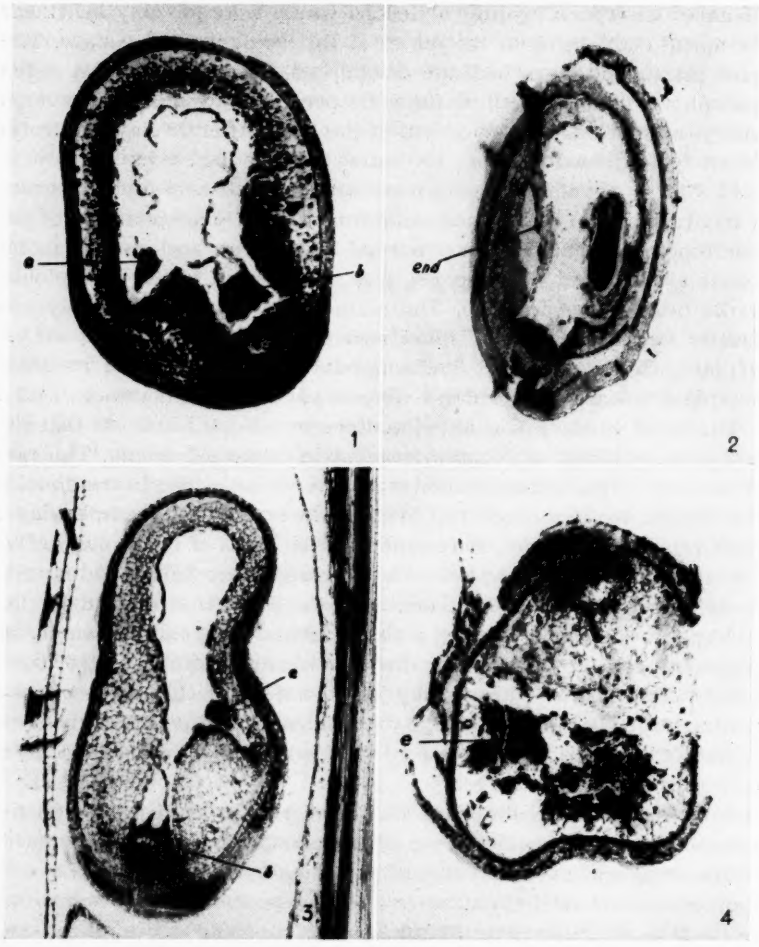


Fig. 1: *B. rapa* 2X×2X. Seed 6 days after pollination. — 2: *B. rapa* 2X×2X, 10 days after pollination. — 3: *B. rapa* 2X×4X, 10 days after pollination. — 4: *B. rapa* 2X×4X, 28 days after pollination, endosperm degenerated, heart embryo.

e=embryo, end=endosperm, b=basal endosperm.

seeds were comparatively small with some starch in the inner layers of the inner integument. The embryo was always a globe of varying size, for instance, with 10—15 cells along the diameter; it never, however, had attained the heart stage. The endosperm was weak, a cytoplasm with nuclei being near the wall of the cavity in the ovule. The basal endosperm contained little cytoplasm and few nuclei; some nuclei could be large. Most ovules had no apical endosperm. However, a few siliqua had better ovules with an endosperm of more normal size and differentiation.

After 14 days such vigorous seeds were larger and had more starch. The embryo had grown and could have small cotyledons, showing in one or two ovules early stage III. The endosperm in these seeds had a large central vacuole and several layers of nuclei, which may form clusters. The endosperm nuclei were rather crowded, indicating a fairly strong mitotic activity with subnormal growth of cytoplasm. In some cases vacuoles indicated initiated cell formation in the endosperm. By far the largest number of seeds had endosperms with no central vacuole, the endosperm being squeezed between the sporophytic tissue of the seed. Though the squeezed cytoplasm is very dense it is not dead; simultaneous mitosis may occur in parts of the endosperm. The collapse of the endosperm is not the result of an active growth of the sporophytic tissue: on the contrary, the latter shows subnormal development. Enlarged endosperm nuclei may occur. Often no embryo could be observed. Embryos with a short suspensor and largely surrounded by sporophytic tissue were observed.

After 21 days the better seeds had an embryo approaching the torpedo stage; the endosperm consisted of a sometimes considerable endosperm with free nuclei, a number of the latter being enlarged. In smaller seeds endosperm and embryo had disapperad. After 28 days the best seeds also were very small, but an embryo may be present; this consisted of a larger globe or had small cotyledons. Endosperm is often absent. Fig. 4 shows a seed with no endosperm but with much sporophytic tissue, an embryo being still distinct. On the other hand, many seeds had a nuclear endosperm with much cytoplasm and nuclei of different size. The basal endosperm may be considerably enlarged. Seeds which were not completely degenerated always contained, however, sporophytic tissue with starch; the endosperm, if present, was abnormal and had not been able to disintegrate these parts of the ovules.

4X×2X. — After 1 day the pollen tubes had only rarely penetrated into the ovules; the first endosperm mitosis and two endosperm nuclei

were, however, observed. After 3 days many ovules may be fertilized; most of them had 16 (4—32) endosperm nuclei. The egg was either elongated or a two-celled proembryo had been formed. Already much starch was assembled in the inner integument, more than in the other crosses. Starch grains may also occur in the embryo sac, but only if fertilization had failed. After 6 days the ovules in general were considerably smaller than in 4X×4X. The widening of the embryo sac was slow and as a rule irregular or incomplete; it had failed in the lower part and here, on the contrary, the chalaza-directed arm is cut off from the rest of the endosperm (Fig. 5). This must be detrimental to the subsequent development. Though the endosperm in 4X×2X is usually very small, cells are formed very early, the apical endosperm being cellular after 6 days. Often the endosperm had already disappeared, the cavity in the ovule being only a fissure. In some seeds the embryo was of unexpected size, consisting of 40—50 cells. Most embryos were small, however.

After 10 days the endosperm in larger seeds was a layer of cells surrounding a rather small central vacuole. The apical endosperm surrounding the globe embryo was cellular. After 14 days exceptionally good seeds occurred in one series of fixations. The embryo in such seeds had a large hypocotyl and very small cotyledons. The endosperm was cellular but its volume was small; it was usually present in only that part of the seed which contained the embryo (Fig. 6). There was no or a very small or degenerated basal endosperm. In most siliqua the seeds were collapsed. The sporophytic tissue near the chalaza region is intact and forms rows of cells with dense cytoplasm (cf. Fig. 11).

After 21 days the seeds have, as a rule, a degenerated endosperm, an embryo globe and sporophytic tissue with much starch. Embryos with very small cotyledons are more rare; one large seed, however, had an embryo with »walking-stick» appearance. After 28 days the content of most seeds was completely degenerated. One embryo of sickly appearance had cotyledons of equal size with the hypocotyl. Some seeds had a small quantity of endosperm; also basal endosperm was found, and this now contained enlarged nuclei.

Conclusions. — There are, as indicated, three time periods in the post-fertilization development. During the first period the largest increase of volume is shown by the sporophytic tissue, particularly the inner integument, much starch being also stored here. This period lasts about 10 days. During the second period the endosperm shows the most vigorous development whereas the inner integument diminishes as the

endosperm enlarges, no starch being stored in the endosperm. The third period shows a rapid and considerable growth of the embryo, which, 21 days after pollination, then fills the seed. The rapidity is largely influenced by external factors. In 1955, 2X×2X after 6 days of bad weather conditions only showed proembryo; after 10 days the embryo was still at the globe stage. Tetraploid siliqua have about the same number of ovules as the diploid, but the ripe siliqua contain on an average only 7—8 seeds as against 19 in the diploid. The cause of this lowered seed fertility of the autotetraploid was largely due, as described above, to degenerations after fertilization.

Seed abortion after 2X×4X and 4X×2X pollinations was not due to absence of fertilized ovules. The initial development was fairly normal, but soon slowed down and was finally interrupted. The development was different in the reziprocal pollinations. In 4X×2X the sporophytic tissue is smaller, but has more starch than normal; the endosperm is very small and poor in cytoplasm but becomes cellular considerably earlier than in normal cases; and the young embryo shows some abnormal traits, only rarely reaching the cotyledonar stage. A peculiar trait is that the chalaza-directed arm of the embryo sac is cut off. The basal endosperm, if present, is weak; it cannot come into closer contact with the underlying tissue, which has a striking cell arrangement. In 2X×4X the initial growth of the sporophytic tissue was better though there was less starch. The endosperm shows much cytoplasm but cell formation is delayed, usually failing completely. Basal and apical endosperm are better here. There are indications of disturbed mitosis in the endosperm, enlarged nuclei gradually becoming more common.

Many seeds degenerate very early in the reciprocal pollinations, the endosperm disappearing and the ovule collapsing before any special developmental traits are shown. Occasional large normal embryos are perhaps diploid or tetraploid; it was, however, impossible to determine the chromosome number of embryo or endosperm. Most triploid embryos do not attain the stages of outer differentiation, the heart stage being only rarely found. This differentiation may, it would seem, be delayed, the globe becoming abnormally large.

IV. BRASSICA OLERACEA

2X×2X. — After 1 day, no ovule with endosperm nuclei was found, only a few being fertilized. After 3 days, as many as 32 endosperm nuclei occurred but mostly a lower number, often only 4—8. The egg

had not divided. After 6 days, most seeds had a proembryo; a lesser number had an embryo globe; and the largest showed the octant stage. Thus, initial embryo development in kale is slow. This is also true of the endosperm. The basal endosperm was, however, more prominent in kale; it had more cytoplasm, and its nuclei were already enlarged. One or two very large endosperm nuclei were also observed quite close to the embryo. Some starch was now for the first time visible in the ovules. After 10 days the quantity of starch of the sporophytic tissue was considerable. The embryo had still not attained the heart stage. The endosperm was nuclear.

After 14 days the embryo was small in most seeds, showing stage III, and rarely approaching torpedo form. The endosperm in most seeds was nuclear, only more rarely had cell formation been initiated. The central vacuole was large. The basal endosperm was very conspicuous with enlarged nuclei in a dense cytoplasm. Isolated normal-sized endosperm nuclei in its vicinity were also surrounded by dense cytoplasm forming spherical, naked cells. After 21 days the embryo fills most of the seed, having grown very rapidly. Smaller embryos, for instance $^1/_4$ of the seed, were also observed. After 28 days the seeds are all filled with an embryo, though some sporophytic tissue may remain within the testa.

4X×4X. — Seed development here was very similar. After 6 days octant embryo was seen, and after 10 days the embryo was a globe. Apical as well as basal endosperm was large. Here also the nuclei of the latter early increase in size. The rate of development varied greatly. After 28 days larger seeds were filled with a full-grown embryo. There were, however, many small seeds containing a small degenerated embryo and some degenerated endosperm as well as sporophytic tissue. — A small number of ovules had no embryo sac, only degenerated macrospores within the intact nucellus.

2X×4X. — After 1 day the siliqua had only a few, after three days many fertilized ovules. Initial seed development was normal. After 6 days the ovule had a proembryo; more infrequently a small globe with 2—8 cells had been formed. The endosperm also was as in 2X×2X. Starch storage seemed somewhat delayed, there being larger quantities only after 10 days. The embryo was then a globe smaller than in 2X×2X, and having only 6—10 cells along its diameter, including dermatogen. Some siliqua had larger seeds, endosperm with many nuclei surrounding a central large vacuole, and large basal and apical endosperms, the former showing enlarged nuclei. There was often a third assembling of nuclei on the top of the endosperm cavity. More often,

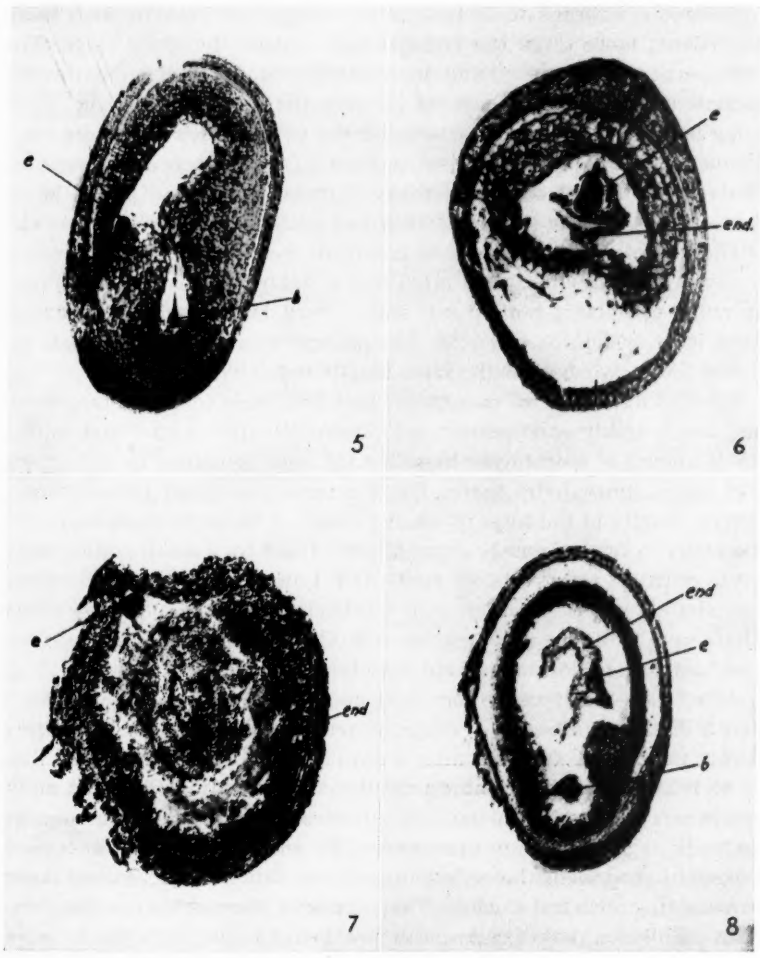


Fig. 5: *B. rapa* 4X×2X, 6 days after pollination. — 6: *B. rapa* 4X×2X, 14 days after pollination. — 7: *B. oleracea* 2X×4X, 21 days after pollination, embryo aborted, endosperm with strongly enlarged nuclei. — 8: *B. oleracea* 4X×2X, 10 days after pollination.

however, the ovules were small, more resembling a 2X×2X after 6 days.

The more vigorous seeds had, after 14 days, an embryo with small cotyledons; more often the embryo still showed the globe stage. The endosperm was nuclear; and in numerous small seeds much endospermic cytoplasm was squeezed between the sporophytic tissue, there being no central vacuole. The peculiarities of the endosperm were more distinct after 21 days. The basal endosperm then generally showed excessive growth and contained many very large nuclei (Fig. 7). Many nuclei were no doubt polyploid; they had probably undergone some sort of endomitosis. Other nuclei had enlarged, seemingly polytene chromosomes. The main endosperm often had a sickly appearance with much granular cytoplasm but no cell walls. Very large nuclei of irregular form indicate fusions of nuclei. The embryo was small; the largest observed had cotyledons of the same length as the hypocotyl.

After 28 days the still small seeds had a variable content. Many seeds had much sickly endospermic cytoplasm with free nuclei and only a small amount of sporophytic tissue. Other seeds contained no endosperm and much sporophytic tissue. Certain seeds contained cellular endosperm, usually in the form of small patches of tissue in the sporophytic tissue, but a few, curiously enough, were filled by a small endospermic tissue with no embryo. Most seeds had, however, an aborted embryo, sometimes a globe, but often with cotyledons which may be very short. These small embryos may be surrounded by some cytoplasm. One larger seed had a more normal embryo with longer, slender cotyledons.

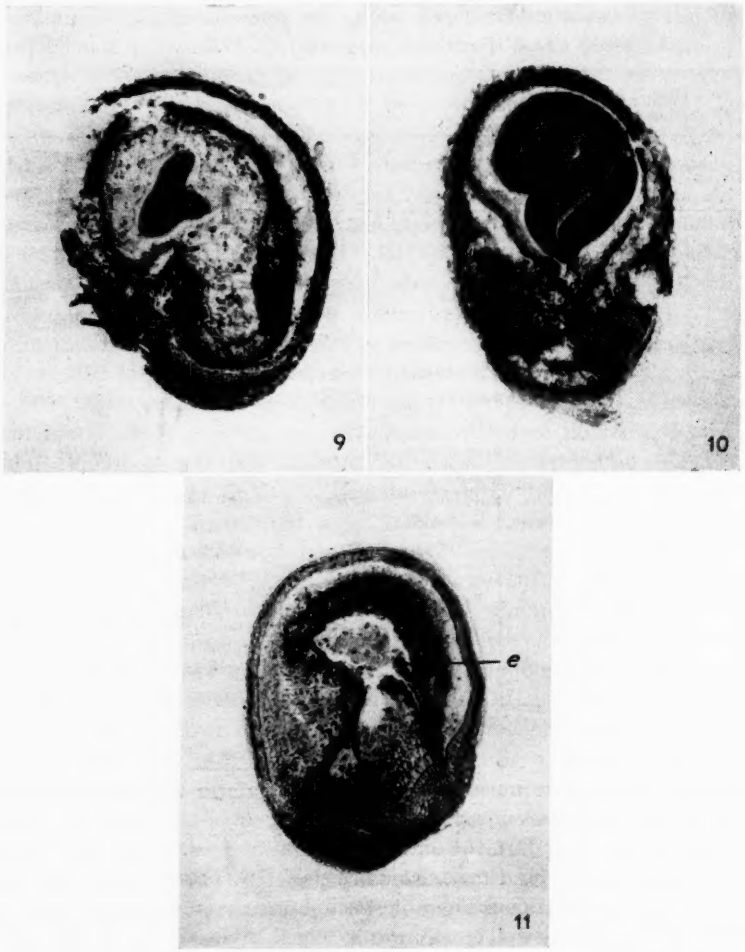
4X×2X. — No fertilizations had occurred after 1 day, but after 2 and 3 days a number of developing ovules were observed. They grew slower than in 4X×4X, but after 6 days the embryo sac was wider than in 4X×2X turnip. The embryo then showed a normal size; the endosperm was weak. After 10 days an apical endosperm usually surrounded an embryo globe with a diameter of 6—10 cells, or sometimes even larger. In good seeds the endosperm was already cellular, several layers surrounding a central vacuole. The volume of the endosperm was, however, small, the basal endosperm not being large (Fig. 8). In other ovules cell formation only had been initiated. The ovules contained much starch.

After 14 days the embryo was often considerably enlarged having cotyledons and showing stage III or IV. The endosperm was small and cellular, the nuclei of the basal endosperm being somewhat, but not much, enlarged. Many ovules are collapsed with no central vacuole.

TABLE 1. *Embryo and endosperm development in turnip and kale; an approximate summary. Upper rows, embryo, lower rows, endosperm. 6—8 d. signifies number of cells along the diameter of a globe embryo. I—VII embryo stages* (*cf. p. 375*).

Days after pollination	3	6	10	14	21
turnip					
2X × 2X	proembryo	I, 6—8 d.	IV, (V)	VI,w.-stick	fills the seed
	32—64 n.	nuclear	beg. cellular	cellular	disappeared
4X × 4X	»	»	»	»	nearly disap.
2X × 4X	proembryo	—	I, 10—15 d.	I—III	I—(IV)
	32	nuclear	nuclear	nuclear	nuclear, degenerating
4X × 2X	egg, proembryo	I, 2—10 d.	I	I—(III)	I—III
	16	nuclear	cellular	cellular	degenerated
kale					
2X × 2X	egg	proembryo-octant	II	III, (IV)	V-nearly fills
	16—32 n.	nuclear	nuclear	nuc., beg. cellular	disappeared
4X × 4X	»	»	»	nuc., beg. cellular	»
2X × 4X	egg	»	I, 6—10 d.	I—III	I—IV
	»	»	nuclear	nuclear, giant nuclei	nuclear, giant nuclei
4X × 2X	»	»	I, 6—10 d.	III—IV	I—VII
	»	»	cellular	cellular	partly disappeared

After 21 days more normal seeds had an embryo and cellular endosperm. Fig. 9 shows endosperm tissue filling the small seed, no central vacuole being left. Here the endosperm contained starch grains, which were assembled around the nucleus of the cells. This was not observed in the other pollinations. There is sporophytic tissue with starch but no basal endosperm here. The embryo is at a fairly early stage. The other seed figured (Fig. 10), is largely filled with a more advanced embryo. It was robust, having a short and thick hypocotyl and thick cotyledons. As a rule, however, seeds still show basal endosperm and much sporophytic tissue. After 28 days the small seeds are to a certain extent filled with an embryo which has an unusual appearance, differing from the normal *Brassica* embryo; it has rather short, thick cotyledons, which



Figs. 9 and 10: *B. oleracea* 4X×2X, 21 days after pollination. — 11: *B. rapa*× *B. oleracea*, 10 days after pollination.

have not grown around the radicle, remaining at the stage of upturned U (VII). Some sporophytic tissue may have been left in the seed. HOWARD reported triploid kale seed to have $^1/_4$ normal seed size. Most seeds are still smaller, having a degenerated or underdeveloped content.

Conclusions. — Seed development, after fertilization, started with a slower rate in kale. Starch also appeared later in the ovule. Generally the basal endosperm was larger than in turnips and its nuclei are early enlarged; there are also large nuclei in contact with the embryo. Later, the development becomes more rapid, the embryo more or less filling the seed after 21 days. The reduction in seed fertility of tetraploid kale has the same causes as that of the tetraploid turnip (cf. p. 377).

The seed fertility after 4X×2X pollinations in kale was much lower than in 4X×4X. The small size of the seeds and the deviating form of the embryo were stressed above. Certain peculiarities of 4X×2X turnip endosperm, that is, its small volume and the early formation of cells, were also distinct in 4X×2X kale. But in contradistinction to 4X×2X turnip the embryo sac with endosperm nuclei had widened in a more normal way after 6 days. The pecularities of 2X×4X endosperms observed in turnip were still more pronounced in kale. These peculiarities are the remaining of the endosperm at the nuclear stage, cell formation occurring late or not at all, the often excessive growth of the basal endosperm, and the high frequency of very large nuclei. The differences between kale and turnip endosperms after 2X×4X pollinations are in conformity with a general difference of the endosperms of turnip and kale. The endosperm of kale has generally more cytoplasm, more basal endosperm and earlier size increase of its nuclei than the turnip endosperm.

V. BRASSICA OLERACEA×B. RAPA

Siliqua obtained through reciprocal pollinations between the investigated species were also investigated. The crosses had been made at the diploid and tetraploid levels. Such pollinations very rarely result in hybrids. It is true that some germinable seeds are formed but most of them contain a matroclinous embryo (cf. OLSSON, JOSEFSSON, HAGBERG, and ELLERSTRÖM, 1955). The appearance of matroclinous plants after *Brassica* crosses has been reported by several authors (for literature, see NISHIYAMA and INAMORI, 1953). Their origin has been interpreted as pseudogamy or as haploid parthenogenesis followed by doubling the chromosomes. The latter explanation was considered more probable. It

is true that such a doubling has often been postulated but so far cytological verifications have not been reported.

Probably matroclinous embryos are only formed in embryo sacs of ovules with a penetrated pollen tube; the polar nuclei were probably fertilized. However, detection of such occasional cases of pseudogamy through embryological-cytological methods is hazardous. My studies were not successful in this respect.

Seed development showed a somewhat different aspect in 1954 and in 1955. In 1954, the development of many ovules was vigorous but unfortunately collections later than 10 days after pollinations were not made. After 10 days, many seeds in 2X turnip×2X kale as well as in 4X turnip×4X kale had a fairly large endosperm with central vacuole and an embryo with short cotyledons; development apparently was normal though somewhat slower than in 2X×2X and 4X×4X turnip, respectively. This was also valid for 2X kale×2X turnip and 4X kale× 4X turnip, which were compared with diploid, and tetraploid kale, respectively. The endosperm was nuclear in kale×turnip after 10 days and the embryo showed globe stage, while after the reciprocal pollinations, turnip×kale, the endosperm could be partly cellular and the embryo show heart stage or stage IV. The abortion of the seeds must occur later than after 10 days.

As already indicated the siliqua fixed in 1955 had grown under more unfavourable weather conditions. All fixations showed delayed fertilizations and slow development of endosperm and embryo during the first time after fertilization. Development after interspecific pollinations was still slower and less vigorous than in controls, the number of unfertilized ovules being considerably larger. Only the observations made on siliqua, fixed 10 days after pollination or later, are reported here.

Kale×turnip. — Seed development after the pollinations 2X kale× 2X turnip and 4X kale×4X turnip was similar. After 10 days fertilized ovules were large but did not contain appreciable quantities of starch; the endosperm was a cytoplasm with comparatively few free nuclei and a large central vacuole. The basal endosperm was small while a dense apical endosperm surrounded the embryo, which was only a proembryo or a small globe with a few cells. Control fixations of kale×kale had an embryo of similar size but a larger endosperm with many more nuclei and a very distinct basal endosperm; the sporophytic tissue contained starch.

After 14 days the embryo globe was enlarged and the apical endosperm distinct; but the main endosperm had undergone few changes

and the basal endosperm was still small. In kale×kale the latter was much larger and had partly penetrated into the adjacent chalazal tissue. After 21 days the embryo globe was larger but only in a minority of seeds showed heart stage; cotyledons only now made their appearance. The endosperm was in most cases a cytoplasm with rather sparse free nuclei which now are often enlarged. These enlarged nuclei contain several vacuoles, their content of chromatin having perhaps not increased. The cavity containing the endosperm with its central vacuole is often rather large; however, in some seeds it was narrow, the endosperm having disappeared and a globe embryo being left. After 28 days the number of seeds with degenerated endosperm increased. The embryo was small with no or very small cotyledons. Peculiar were the small changes of the sporophytic tissue in these hybrid seeds during the latter part of development. It was large, usually not diminished by the endosperm; it had little starch and little differentiation; and no testa structure was distinct. Thus it had a very juvenile appearance.

Turnip×kale. — The ovules examined after 10 days were considerably smaller than in the reciprocal combinations. The sporophytic tissue of the ovules contained much starch, more than in the controls. The cavity within the ovule had very varying width. Sometimes its chalaza-directed part was cut off as in 4X×2X turnip. In either case the endosperm is here very weak; there was usually no basal endosperm or the latter had only a few nuclei; and the main endosperm had little cytoplasm and rather few nuclei. However, cell formation was often initiated near the small embryo.

After 14 days the endosperm, if present, was cellular with comparatively few large cells, which did not show any regular »arrangement». The embryo was a globe with, for instance, 10 cells along the diameter. Very often the endosperm had disappeared, leaving the globe embryo in the cavity. Sometimes the cavity was only a fissure and no embryo was left. After 21 and 28 days all investigated seeds were very small; they had a degenerated or dried endosperm; and the embryo, if present, was a globe or showed stage II. The sporophytic tissue contained much starch.

Turnip rape×kale. — Development here was similar to turnip×kale. Already after 66 hours there was much starch in the ovules, the number of endosperm nuclei being now 4—32. After 6 days the endosperm seemed starved with no distinct basal or apical parts. A seed 10 days after pollination is shown in Fig. 11. The cavity in the small ovule is divided, its chalaza-directed part being cut off. The endosperm consists

of a very loose, incomplete tissue with no central vacuole. There is no basal endosperm but a few endosperm cells in the smaller cavity near the chalaza. The embryo is distinct. The embryo was a globe still small. Often the embryo suspensor was curved. Though many seeds were larger with a larger cavity, their endosperm was similar: cellular, lacking central vacuole and lacking basal endosperm. After 14 days, degenerations were frequent and after 21 and 28 days an embryo, showing stage I or II, was sometimes left surrounded by a sporophytic tissue filled with starch.

Turnip rape development is similar to development in turnip, but in 1955 was somewhat slower. After 21 days there was still endosperm tissue in the seed, and starch grains could be observed in endosperm cells, usually assembled near the nucleus of a cell.

Conclusions. — Pollinations between *B. rapa* and *B. oleracea* more or less often lead to fertilizations. The initial development of endosperm and embryo is normal but soon certain peculiarities may be observed. In 1955 degeneration was rather rapid, later than 10 days after pollination. The hybrid seeds were different in the reciprocal pollinations. With kale as mother plant the seeds were larger, and contained little starch; the endosperm remained nuclear; and embryo may show the heart stage. With turnip or turnip rape as mother plants hybrid seeds were small with much starch; and had a small, early cellular endosperm with no central vacuole; they usually had no basal endosperm and degenerated rapidly; and the embryo never attained stages with visible cotyledons. In 1954 the hybrid seeds had larger endosperms but certain reciprocal differences were also evident that year.

VI. GENERAL CONCLUSIONS

An important cause of seed abortion and embryo lethality is deficient endosperm (BRINK and COOPER, 1947). The endosperm is not only a storage tissue which is used at seed germination, but it also contains at earlier stages substances necessary to the nutrition and growth of the embryo within the seed; the latter is its sole function in angiosperms with non-endospermous seeds. The importance of the endosperms in these respects is demonstrated by the influence of cocosmilk on successful embryo growth *in vitro*; a stimulative effect of *Hordeum* endosperm has also been shown (ZIEBUHR and BRINK, 1951). The developing endosperm also probably influences the sporophytic tissue of the ovule; the observations made in *Brassica* indicate that a good endosperm is

necessary to normal mitotic activity in these tissues; on the other hand, the endosperm later initiates their destruction. Here the stored integument starch is of importance during the stages of rapid embryo growth.

Disturbed endosperm development generally takes place after crosses between diploids and their autopolyploids. It has been pointed out that the aspect of the endosperm is different in the reciprocal crosses (see HÅKANSSON and ELLERSTRÖM, 1950; HÅKANSSON, 1953). In a number of instances 2X×4X pollinations have resulted in an endosperm with delayed or inhibited formation of cells and storage of starch, a strong tendency to mitotic irregularities, and a good deal of cytoplasm. On the other hand, the endosperm after 4X×2X has generally a small volume, and shows early formation of cells and storage; mitotic irregularities have only rarely been mentioned. Similar contrasting differences in the reciprocal crosses were also observed in *Brassica rapa* and *B. oleracea*, indicating their general occurrence. Mitotic disturbances in 2X×4X were indicated by the high frequency of enlarged nuclei.

Recently, STOKES (1955) investigated a case of seed sterility in a Crucifer where disturbed endosperm development had been observed, namely in the horse-radish, *Armoracia rusticana*. It rarely sets seed, owing to lack of pollen and self-incompatibility; but as STOKES showed, this was also due to fairly frequent degeneration of developing seeds. He obtained a number of fertilizations after crossing certain strains. They occurred 18—30 hours after pollinations and the further seed development also evidenced in normal cases about the same rates of development found here in turnip and kale. As the most frequent cause of seed failure, the same author stresses the failure of endosperm to function properly; more rare was embryo mortality in a normal endosperm. Similar to earlier workers he assumes the cause of endosperm degenerations to be »a physiologic upset between the endosperm and the maternal tissue». As an indication of this, STOKES noted the relation between the basal endosperm and underlying »nucellar tissue», where breakage products occurred between the endosperm and the latter tissue.

The importance of the basal endosperm in the nutrition of the seed is clear. In sterile crosses it is generally smaller and is unable to penetrate into the underlying tissue, which remains fairly intact. In the 4X×2X turnip a degenerated basal endosperm lies on the strongly developed underlying tissue, which consists of rows of small cells rich in cytoplasm. »Breakage products», as evidence of disturbed physiology, were, however, hardly observed. Also a very large basal endosperm may be

ineffective as is seen in the 2X×4X kale. A normal apical endosperm seems to be of importance in the nutrition of the young embryo, but in seeds with interrupted embryo development it is absent or weak. Its importance is clear from the fact that embryos with abnormally short suspensor and therefore only surrounded by sporophytic tissue do not develop further. It seems that the general significance of the elongation of the fertilized egg and the formation of a suspensor lies in the removal of the embryo from the very narrow micropylar end of the embryo sac to make possible a development surrounded by endosperm.

Embryo development soon slows down in the seed-sterile crosses and is interrupted. The development follows the usual scheme but the embryo is sometimes abnormal, the globe becoming large before the cotyledons begin to appear. Only more rarely does the embryo attain or approach the torpedo stage. This probably means, however, that in rare cases it may be possible to attain triploid turnip seedlings through *in vitro* growth in the manner described by RIJVIN. It is a general rule, even in cases of complete sterility such as in *Galeopsis pubescens* (HÅKANSSON, 1952), that 4X×2X crosses result in more advanced embryos than the corresponding 2X×4X. This is, however, not valid in *Brassica rapa*, probably because of a secondary factor, namely that here the embryo sac with endosperm does not widen in its chalaza-directed part. This must result in insufficient nutrition of endosperm and embryo.

A few seeds of more normal size, containing a normal, large embryo, had been formed. As maternal diploid or tetraploid plants occasionally appear in the crosses, it seems probable that such large embryos are not triploid; this could not, however, be verified through chromosome countings. On the other hand, the siliqua contained a varying number of very small seeds, whose development had been interrupted soon after fertilization. Such early seed abortions also occur in 2X×2X, though they were more frequent in 4X×4X. Presumably the nutrient supply to the siliqua is insufficient to allow the development of all fertilized ovules into mature seeds.

The observations are consistent with the view of a central rôle of the endosperm during seed development and the importance of a normal exchange between the endosperm and the sporophytic tissue of the ovule. The disturbed exchange leads to starvation and abnormal development of the endosperm. MÜNTZING was the first to point out that after crosses between plants with a different number of genomes the normal chromosome ratios of embryo : endosperm : sporophytic tissue 2 : 3 : 2

is changed; for instance, the ratio endosperm : sporophytic tissue is after 2X×4X pollinations 4 : 2, after 4X×2X 5 : 4. This must influence the normal physiological exchange.

Several other explanations of the causes of endosperm degenerations have been put forward. Such are somatoplastic sterility in the older meaning of this term, namely an active growth of the somatic parts of the ovule encroaching on the endosperm, and the forming of »tumours» in the endosperm cavity; but these explanations cannot be valid here. Out of the question also is an abnormal development of the antipodals, which have been thought to influence endosperm mitosis. Recently, BROCK (1954, 1955) has shown that in seed sterile *Lilium* and *Hyacinthus* hybrids endosperm failure and subsequent embryo abortion occur. Some time after fertilization chromosome bridges begin to appear in the endosperm, the mitotic disturbances soon increase in frequency, and this ends in the degeneration of the whole endosperm. In *Ranunculus auricomus*, which is pseudogamous, RUTISHAUSER (1955) reports that seed sterility occurs »sogar in Kreuzungen zwischen Mikrospezies derselben Sammelart wegen des Vorkommens spontaner Chromosomenbrücke im Endosperm». In *Brassica* such disturbances are hardly the primary cause of endosperm degeneration. Mitotic irregularities, it is true, could not be closely studied here, but clearly they lead to the forming of polyploid nuclei or nuclei with »giant» chromosomes. However, they appear rather late, mostly in 2X×4X, and seem here to be rather a secondary than a primary disturbance. HOWARD (1939, 1947) ascribed seed abortion in crosses between diploids and tetraploids in *Brassica* and *Nasturtium* to changed ratios between embryo and endosperm. However, embryological observations hardly favour such a view though conclusive demonstration of its fallacy is difficult.

Seed development after reciprocal pollinations between *B. oleracea* and *B. rapa* also showed differences. A greater similarity to the mother plant was discernible. Certain peculiarities observed in kale and the kale crosses were found after kale×turnip pollinations, while in turnip× kale traits recalling the turnip crosses were observed in ovule growth and endosperm. It is perhaps merely a coincidence that kale×turnip shows peculiarities of 2X×4X crosses, turnip×kale of 4X×2X crosses. Kale has, it is true, a somewhat lower chromosome number but it seems hardly probable that this is the cause of such similarities. The seed sterility after interspecific crosses must be largely due to qualitative differences between the genomes. VALENTINE (1947) observed differences between the seeds in reciprocal crosses between *Primula vulgaris*

and *P. elatior*, two species with the same number of chromosomes. He pointed out that the maternal parent contributes two genomes, the paternal parent only one, to the endosperm; this may explain the differences in seed quality in reciprocal crosses. An influence from the genotype of the sporophytic tissues of the ovules also seems possible.

SUMMARY

Seed development in *Brassica rapa* and *B. oleracea* shows certain minor differences. It is somewhat slower in the latter species.

After pollinations between diploids and autotetraploids endosperm development is strongly disturbed. Embryo development is more normal, but slows down and is generally interrupted.

After 2X×4X pollinations the endosperm remains nuclear for a long period, cell formation being delayed or inhibited. In the kale cross endosperm cytoplasm often becomes excessive, enlarged endosperm nuclei being formed in great numbers. In the turnip cross the endosperm had less cytoplasm, and the increase in nuclear size begins later and is less frequent.

After 4X×2X crosses the ovules increase in size more slowly than in other pollinations. The volume of the endosperm is small but cell formation is earlier than in 2X×2X and 4X×4X. In the kale crosses a mature embryo is sometimes formed, though it has a somewhat different appearance to a normal embryo. In the turnip crosses the chalaza-directed arm of the embryo sac does not widen, the basal endosperm being cut off, which may explain the unexpected abortion of all embryos.

After interspecific pollinations seed development also becomes strongly disturbed, and as a rule mature hybrid embryos are not formed. The endosperm in kale×turnip remained at a nuclear stage while the smaller endosperm in turnip×kale was early more or less cellular and often lacked a basal endosperm. The endosperms degenerate, the embryo showing somewhat better development in kale×turnip; but as a rule there were aborts in both the reciprocal crosses.

The cause of the embryo lethality is probably the endosperm deficiency brought on by disturbed nutritional relations between the endosperm and the surrounding sporophytic tissue of the ovule.

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RECURRENT RECIPROCAL SELECTION

RESULTS OF THREE MODEL EXPERIMENTS ON DROSOPHILA FOR IMPROVEMENT OF QUANTITATIVE CHARACTERS

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I. INTRODUCTION

THE great success of the hybrid corn breeding has called upon the animal breeders to take advantage of heterosis in animal production as well. So far, the establishment of useful inbred lines for crossbreeding has not succeded, mostly due to low fertility and susceptibility to diseases that make the lines difficult to maintain. Other methods for exploitation of hybrid vigour therefore have been outlined. One of the first was suggested by BONNIER (1939) for the production of crossbred poultry. HULL (1945) evaluated the recurrent selection for specific combining ability in corn; a method which rests on the hypothesis of overdominance and which was later on extended to the so-called recurrent reciprocal selection method (henceforth called RRS) by COMSTOCK, ROBINSON and HARVEY (1949). In this paper and that of BELL, MOORE, BOHREN and WARREN (1952) theoretical comparisons are made between the RRS method and others, including HULL's method of selection for specific combining ability and selection within a closed flock. Different degrees of dominance (also overdominance) are assumed, but the calculations are made under the assumption of no epistatic interaction. The RRS method seems to be equivalent to other methods if additiveness or more or less complete dominance is prevailing and superior in cases of overdominance.

But, to cite BELL *et al.*: »statistical calculations and deductive reasoning cannot establish the real value of any breeding system». Aware of this fact, these authors have started breeding experiments with poultry and model experiments with *Drosophila*, part of which were submitted at the Genetical Congress in 1953; BELL, MOORE and WARREN (1954). Independently, experiments with mice and *Drosophila* were started at the Animal Breeding Institute, Wiad, in 1951 by the present author in

cooperation with Dr. A. HANSSON, in order to test a breeding method for utilizing heterosis in animal breeding, in fact identical to the RRS method. The results of the *Drosophila* experiments will be accounted for in this paper. The investigations have been supported by research grants from the Swedish Agricultural and Natural Science Research Councils.

II. OUTLINE OF EXPERIMENTS

For the investigations wild type *Drosophila melanogaster* from different stocks were used. The animals were reared in bottles with standard medium or in vials on yeast suspension soaked up in cellulose tissue. The breeding temperature was 25° C.

The experimental design is shown in Fig. 1. Two genetically heterogeneous populations, made up by crossing different wild type stocks, were randomly divided in two groups each. From these groups 4 selection lines (I—IV) were started. Selection was made solely between males and based on progeny testing. Lines II and III were selected according to the RRS method. The males from line II were crossed to females from line III and *vice versa*. The progeny testing was made on the crossbred offspring, which were then discarded. Lines I and IV, from the same origin as II and III, respectively, were used as controls, subjected to closed flock selection, and their males were tested on offspring from crosses with females from their own line. All 4 lines were then propagated by the selected males and a random sample of females from their own line and the next cycle of selection was started from this purebred offspring.

Three experiments were performed where selection was based on different quantitative characters. In experiment 1 egg production was studied as measured by the mean daily egg number during the 5th to the 9th day of living of the females. Experiment 2 started when exp. 1 was terminated after 20 cycles of selection, and the selection criterion was hatchability of the eggs. It was continued for 13 selection cycles. In experiment 3 the lines were selected for high body weight. As the breeding method was found to be unsuitable for the selection of this character, this experiment was only kept going during 6 cycles.

The breeding method was identical in exp. 1 and 2. From each line 16—20 males were taken out for testing. They were put separately in vials together with 3 virgin females for mating. In lines I and IV the females were taken from the male's own line, and lines II and III were reciprocally crossmated, according to the scheme. The vials were supplied

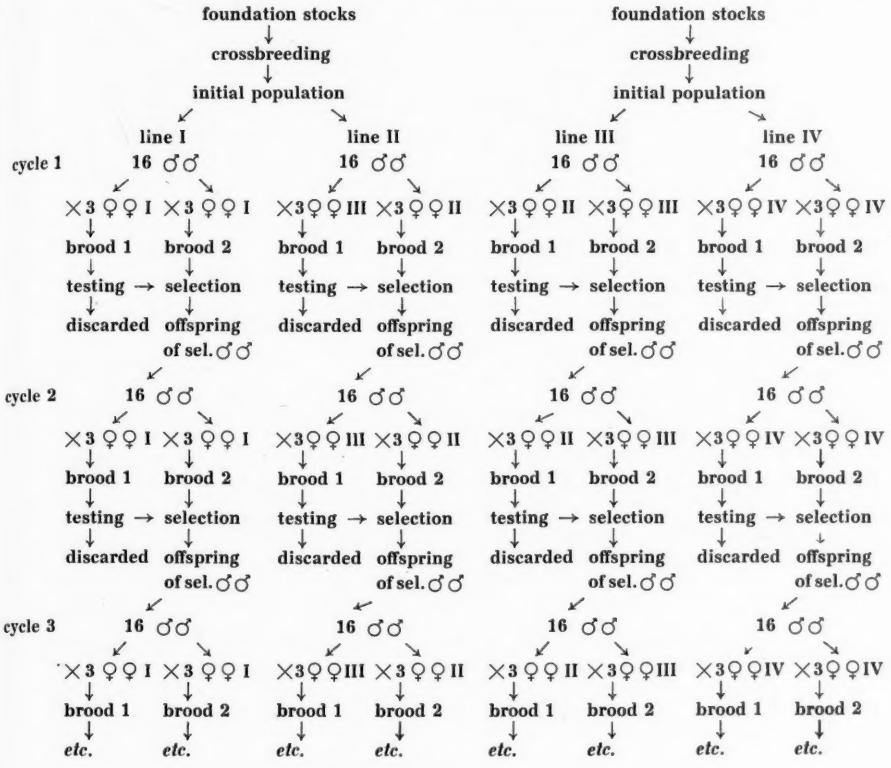

Fig. 1. Outline of the selection experiments.

with a cut-out square of standard food seeded with yeast and placed on a romboid piece of cardboard. After 3 days, when mating was supposed to have taken place, the males were transferred to other vials and mated to a fresh set of 3 virgin females, this time in all cases from the same line as the males. From the offspring of the first mating 4 daughters were taken out after each male for testing. They were put individually in vials each together with one male (usually a brother). In exp. 1 all the vials were recorded for eggs and larvae and those females that gave viable offspring had their egg production recorded during 5 days starting with their 5th day of living. During these days they were supplied with squares of food blackened with charcoal which were exchanged

daily. The number of eggs deposited on the black food was counted at 20× magnification, whereafter the pieces of food were discarded. If a male died during the testing period another was supplied, if a female died before the end of the testing period her record was excluded.

In experiment 2 the daughters were supplied with black food usually during 3 consecutive days. Their deposited eggs were kept for 28—30 hr after counting and then checked for hatching. As all hatching is known to occur within 24 hr this interval was sufficient for a recording of the total hatchability. The empty egg shells could easily be distinguished from the eggs that had not hatched. The percentage of hatchability was calculated on the total numbers. When the egg number was less than 30, or when no eggs hatched, the values were discarded.

No progeny was raised from the testing females. When the testing period was ended the mean performance was calculated for each group of common paternity and on account of these the best 4 of the fathers were selected to propagate the lines. As these had earlier been mated a second time their purebred offspring from this second mating was ready to be used in the next cycle of selection at the time when the progeny test was finished. By this breeding method the time used for each cycle was reduced to 21 days in exp. 1 and 2. The lines were prevented from strong inbreeding by using 3 females to each male and by mixing the offspring from the 4 selected males before starting a new cycle of selection. In some cases one generation without selection was inserted by massbreeding this offspring and taking out males for testing from the next generation.

Such as the experiments were designed it was not possible to take into consideration the individual merit of the females either in the crossbred or in the control lines. It was intended to save $^1/_4$ of the tested males for propagation of the lines but some of the males died before the second mating had taken place or failed to give offspring in one of the matings. Such males could not be used for selection and thus the strength of selection was decreased and also unequally distributed between the lines. When records from the progeny were available they were included in the comparisons between lines even if the male had failed to give a second brood.

The breeding method was modified in experiment 3, where body weight was the selection criterion, as this character is strongly influenced by culturing conditions. From the first brood of the males to be tested 25—30 newly hatched larvae were picked out and placed in vials containing yeast suspension soaked up in cellulose tissue. This was

ment to give optimal developmental conditions, equivalent for all vials. Preliminary tests showed that the imagos changed their weight appreciably from one day to the next, males less than females. The weighing was therefore undertaken with 24 ± 4 hr old animals using a precision balance. The flies were weighed individually and the means for males and females were calculated separately. The value of the fathers was judged on account of the grand mean of their male and female offspring, whereafter the second broods of those selected for propagation were mixed and used for the next cycle of selection.

III. RESULTS

1. Selection for increased egg production

The populations from which this selection experiment started were made up by crossing together the laboratory stocks *Oregon* and *Stäket* (origin of lines I and II) and the inbred stocks *Örebro* and *Florida* (origin of lines III and IV). For preliminary testing, records were made of the egg production of females from these four foundation stocks and the F_1 and F_2 from their crosses. Such tests were also repeated at some occasions later on during the selection experiment. The combined results of these tests and analyses of variance are shown in Table 1. The foundation stocks gave different number of eggs and so did the reciprocal crosses in F_1. The differences between reciprocal crosses were not maintained in F_2 and thus were probably due to some delayed influence from the parental stocks. F_2, on the other hand, showed a significant difference between the two crosses of different origin, which suggests a genetically conditioned difference in egg production. Further, the variation between the total records of the females are significantly greater than the daily fluctuations for individual females.

It is also clear from the tests that egg production is a character which is strongly influenced by heterosis. The egg number in F_1 is much higher than in the foundation stocks and also higher than in F_2. However, the most significant fluctuations of all are those found between time of year. If these fluctuations are of annual recurrence cannot be stated, as the tests were not repeated the following year, but anyway they must be of major importance for the outcome of the selection experiment.

In the first cycle of selection the males to be progeny tested were taken out from the F_2 populations, where F_2's from reciprocal crosses

TABLE 1. *Preliminary tests of egg production.*

a. Mean daily egg production. n=number of tested females.

Foundation stocks			F_1			F_2		
	n	egg yield		n	egg yield		n	egg yield
Oregon	26	40,8	O × S	30	41,2	O × S	8	47,2
Stäket	28	49,3	S × O	23	81,4	S × O	9	53,3
Örebro	19	31,2	Ö × F	23	39,8	Ö × F	7	34,8
Florida	3	35,5	F × Ö	3	79,2	F × Ö	11	34,0
Mean		39,2	Mean		60,4	Mean		42,5

Month	Foundation stocks		F_1		F_2	
	n	egg yield	n	egg yield	n	egg yield
July	23	13,8	33	26,2		
Aug.			39	62,2	35	42,5
Nov.	22	47,6				
Dec.	22	58,6	7	94,9		
Jan.	9	39,2				

b. Analyses of variance.

	Source of variance	Degrees of freedom	Mean square
Foundation stocks	Between months within stocks	6	35517***
	» stocks within months	6	9030***
	» females	66	1137***
	» daily yields of females	304	225
F_1	Between months within crosses	3	21541***
	» crosses within months	2	44
	» reciprocals within crosses	2	5934***
	» females	72	1228***
	» daily yields of females	316	168
F_2	Between crosses	1	11733***
	» reciprocals within crosses	2	334
	» females	31	688***
	» daily yields of females	140	166

*** $P < 0{,}001$.

TABLE 2. *Mean daily egg production of tested females in experiment 1. n=number of progeny tested males.*

Cycle of selection	Origin of females							
	I		II♂×III♀		III♂×II♀		IV	
	n	egg yield	n	egg yield	n	egg yield	n	egg yield
1	14	37,45	8	42,55	5	20,33	12	34,35
2	15	36,62	12	40,90	9	46,41	10	33,77
3	15	53,62	9	61,32	13	62,33	13	52,19
4	15	55,27	11	61,54	5	51,49	9	45,76
5	8	64,79	9	65,36	4	42,99	9	43,74
6	19	64,10	2	84,71	3	88,56	3	66,89
7	5	60,25	6	58,39	13	75,18	7	62,81
8	12	59,58	7	57,68	3	68,26	3	58,58
9	17	87,25	12	93,62	8	88,06	5	76,03
10	5	76,07	1	63,00	1	57,60	2	41,00
11	9	44,13	2	38,28	3	48,63	3	48,95
12	8	22,30	4	30,74	9	31,77	6	25,57
13	11	29,08	3	42,62	—	—	1	46,60
14	2	45,83	11	39,57	10	42,48	2	41,60
15	13	5,26	15	9,69	15	6,00	16	6,55
16	16	2,76	7	2,64	7	3,21	6	3,02
17	9	85,03	12	87,72	14	86,15	11	57,07
18	12	86,46	1	87,60	7	87,45	8	95,44
19	11	49,82	9	44,52	9	44,18	7	64,85
20	9	56,34	4	52,10	5	34,21	5	42,66
Mean	11,2	51,10	7,25	53,23	7,15	51,86	6,90	47,37

Mean of crossbred females (II ♂×III ♀ and III ♂×II ♀): 52,56 eggs/day/female.
» » control » (I and IV): 49,24 eggs/day/female.

had been mixed. The *Oregon—Stäket* population was randomly divided to form lines I and II, the *Örebro—Florida* population to form lines III and IV. The tests of the four lines were made simultaneously in each cycle of selection during the whole experiment to keep environmental influences as equivalent as possible.

The selection was continued for 20 cycles during a time of $1^1/_2$ year. The mean egg production of the tested females is shown in Fig. 2 and Table 2, and the analysis of variance of the results in Table 3.

The great seasonal variations in egg production is the most striking feature of the figure. The apparent increase in egg production during the first 10 cycles of selection may not be caused by selection itself but

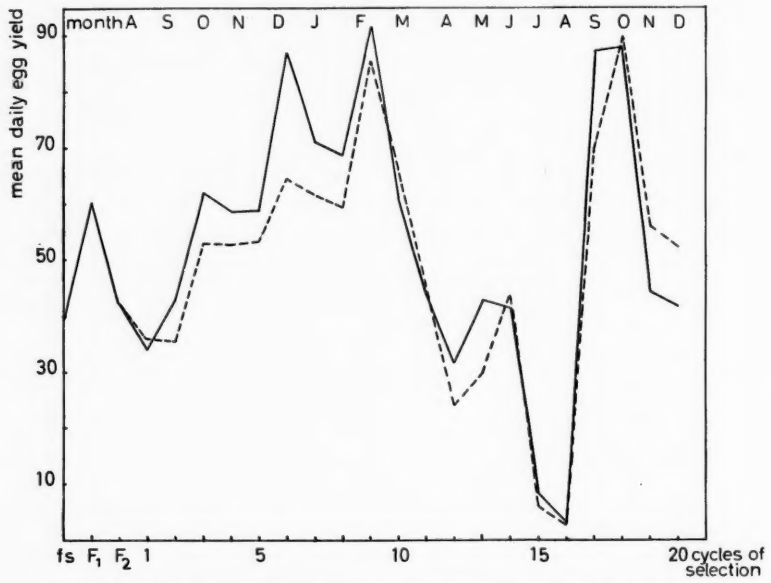

Fig. 2. Result of experiment 1. Selection for increased egg production.
—— crossbred females. - - - - control females.

merely be a reflection of seasonal variations, as during July and August of the second year a minimum is reached which lies below even the production of the foundation stocks. It is thus not possible to prove that selection has been effective in raising the egg production.

A comparison between the performance of crossbred and control females reveals a significant advantage of the crossbred females, amounting to about 3 eggs a day, or 6—7 %. This is the important result of the experiment, which thus confirms the advantage of the RRS method when egg production is concerned.

Significant differences in performance are also found between the control lines, but not between the reciprocally crossbred lines. This is to be expected if the two initial populations have genetically different capacities and if no cytoplasmic or maternal influences are involved that may modify the reciprocally crossbred offspring. The significant differences between groups of common paternity indicate an hereditary component in egg production. This genetical part of the variation within lines and generations, calculated as 4×the variance between groups,

TABLE 3. *Analysis of variance of experiment 1.*

Source of variance	Degrees of freedom	Mean square	Variance ratio
Between lines	3	139318,0	16,0***
Crossbred versus control females	1	196711,0	22,6***
Between reciprocally crossbred females	1	1322,0	—
» control lines	1	210621,0	24,2***
Between generations	19	1692197,0	194,0***
Line-generation interaction	57	34242,7	3,9***
Between groups of females of common paternity within lines and generations	572	8722,1	1,46***
Between females of common paternity	1524	5988,1	
Total	2175		

*** $P < 0{,}001$

amounts to 54 %. As the tested females within such groups are not necessarily half-sisters, but may as well be full sisters this figure is probably too high. The variance between daily records of individual females has not been calculated in the analysis where the total egg yield of each female has been used as variate values.

In Table 2 is also given the number of progeny tested males in each generation and line. It is evident that line I has been at an advantage as its mean number of tested males is higher than in the other lines. In generation 13 no offspring were obtained from the cross III ♂×II ♀, and the propagation was made with untested males.

2. Selection for increased hatchability of eggs

Before experiment 1 was broken off after 20 cycles of selection, the hatchability had been determined for the eggs laid by the tested females in cycle 19. The percentages of hatchability were found to be: line I: 74,1 %, II ♂×III ♀: 67,4 %, III ♂×II ♀: 67,6%, IV: 53,7 %. Analysis of variance showed that as well the difference between crossbred and control females as the difference between the reciprocally crossbred females were insignificant, whereas line I had a really higher hatchability than line IV. The variance between groups of common paternity was insignificant, which might indicate that the lines had become genetically rather homogeneous during the course of selection. As hatchability is less influenced by environmental factors than egg production,

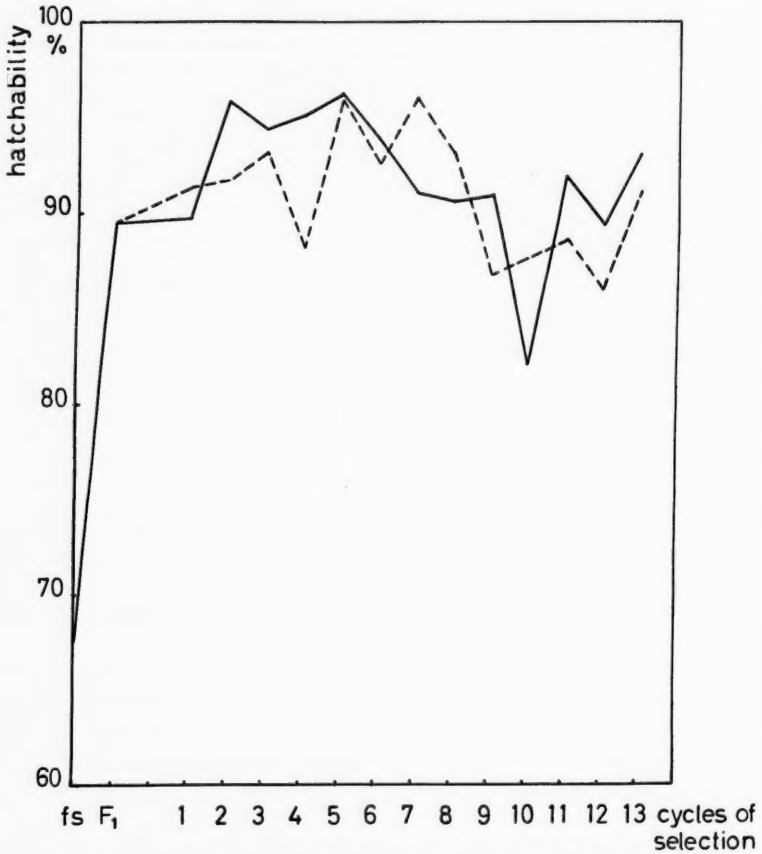

Fig. 3. Result of experiment 2. Selection for increased hatchability.
—— crossbred females. - - - - control females.

it was thought to be a suitable character for selection, and was used as selection criterion in experiment 2.

This was started from two populations made up by crossing together the lines obtained in experiment 1. Lines I and II were crossed together and likewise lines III and IV. These crosses were tested for degree of hatchability and gave 86,2 % and 93,2 %, respectively, a difference that was found to be insignificant, as were also the differences between reciprocal crosses. A comparison with the hatchability of the eggs laid in

TABLE 4. *Mean percentage of hatchability of tested females in experiment 2. n=number of progeny tested males.*

Cycle of selection	Origin of females							
	I		II♂×III♀		III♂×II♀		IV	
	n	%	n	%	n	%	n	%
1	4	94,9	9	90,1	8	89,3	8	89,1
2	12	93,0	8	96,2	10	95,4	7	88,2
3	16	90,1	15	94,7	15	94,2	15	95,5
4	15	84,0	18	97,0	17	92,7	14	93,6
5	13	95,5	15	98,0	16	95,0	14	96,2
6	14	95,9	16	95,5	16	92,0	17	89,6
7	6	97,6	2	87,9	3	92,3	2	90,1
8	7	90,6	13	89,9	14	91,2	10	95,7
9	10	86,8	15	90,3	4	93,3	7	86,5
10	13	88,3	11	76,4	12	87,2	14	86,9
11	10	88,9	1	92,0	7	91,7	4	87,2
12	11	86,5	2	84,5	6	90,8	16	85,7
13	15	92,9	14	93,1	13	93,1	16	89,0
Mean	11,23	91,5	10,69	93,4	10,85	92,6	11,08	91,2

Mean of crossbred females (II ♂×III ♀ and III ♂×II ♀): 93,0 % hatchability.
» » control » (I and IV): 91,3 % hatchability.

TABLE 5. *Analysis of variance of experiment 2 after angular transformation.*

Source of variance	Degrees of freedom	Mean square	Variance ratio
Between lines	3	508,986	1.44
Crossbred versus control females	1	1342,551	3,81
Between reciprocally crossbred females	1	167,521	—
» control lines	1	16,886	—
Between generations	12	1328,571	3,77***
Line-generation interaction	36	352,044	3,87***
Between groups of females of common paternity within lines and generations	518	90,875	1,63***
Between females of common paternity	1163	55,774	
Total	1732		

*** $P < 0,001$.

cycle 19 of experiment 1 shows that heterosis has increased the hatchability with about 22 %.

The selection in experiment 2 started from the F_2 populations which were divided to form the new lines I—IV. This experiment was kept going for 13 cycles of selection. The result is seen from Fig. 3 and Tables 4 and 5. Females giving less than 30 eggs during the testing period were excluded from the calculations to make the percentage values more reliable. The angular transformation has been used as the percentages usually exceed 70 and the distribution thus is likely to be scewed. All means are back transformations from calculations made on transformed values.

The egg hatchability of the females tested in this selection experiment gives an overall mean of 92,2 % which is insignificantly higher than what was obtained in the crosses that made up the initial populations. The effect of selection is thus dubious also in this experiment. Between the 6th and the 7th cycle of selection two month elapsed, during which interval the Drosophila Laboratory and all the stocks were moved to a new locality at Upsala. The vitality of the selection lines was disturbed by the environmental change and they needed some generations of recovery before another cycle of selection could be started. It appears from Fig. 3 that the hatchability became throughout a little lower after the transfer, what may be caused by difficulties of adaption to the new environment.

The most important point also in this experiment is the comparison between crossbred and control females. As the grand means of Table 4 show, the crossbred females exceed those of the control lines. The excess is only 1,7 %, which means a superior performance of less than 2 %. From Table 5 it appears that this difference is not quite significant at the 5 % level (5 % ratio: 4,11), but as a complement to experiment 1 it supports the RRS method.

The differences between control lines and between reciprocally crossbred lines are quite insignificant. The variance between groups of common paternity amounts to 19,95 % of the total variance within lines and generations and the genetical component of this variance may thus be estimated to 80 %, or much more than what was found for the egg production.

3. Selection for increased body weight

In experiment 3 three foundation stocks were used for each population. The wild type stocks *Algeria*, *Stäket* and *Oregon* were intercrossed

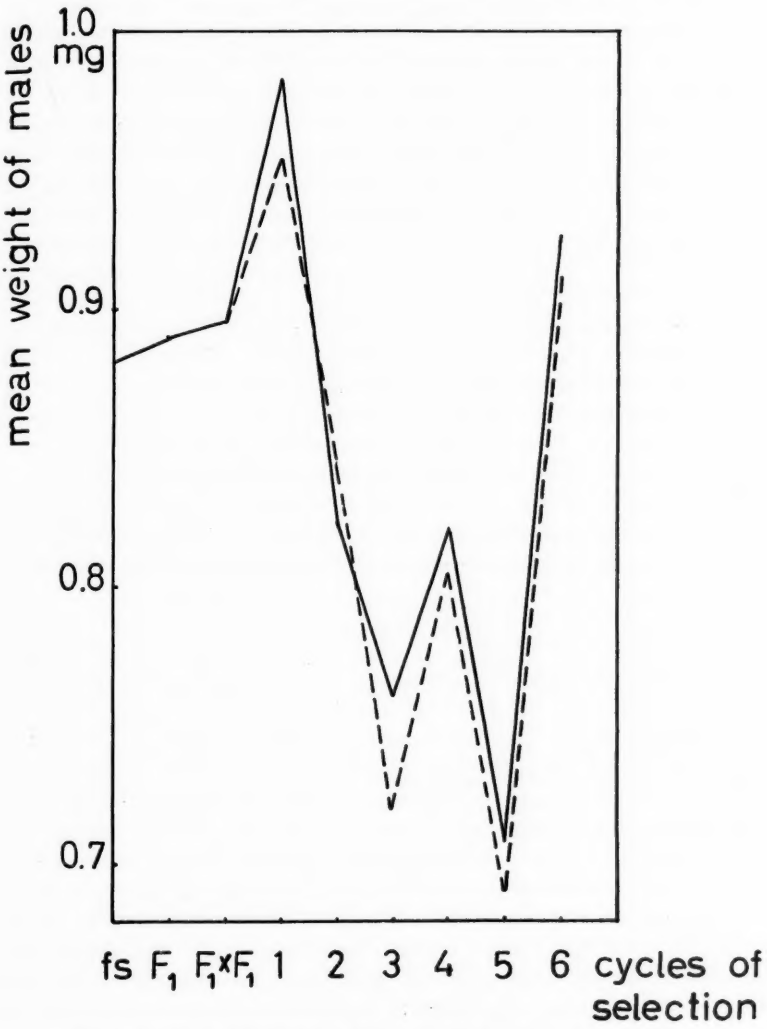

Fig. 4. Result of experiment 3. Selection for increased body weight.
—— crossbred males. - - - - control males.

TABLE 6. *Mean weight in mg of male offspring in experiment 3. n=number of progeny tested males.*

Cycle of celection	Origin of males							
	I		II♂×III♀		III♂×II♀		IV	
	n	weight	n	weight	n	weight	n	weight
1	7	0,969	2	0,970	3	0,994	3	0,938
2	8	0,845	8	0,835	9	0,813	8	0,845
3	10	0,710	10	0,768	9	0,753	9	0,730
4	10	0,796	11	0,826	8	0,814	4	0,852
5	10	0,675	10	0,711	7	0,704	10	0,705
6	11	0,931	4	0,914	11	0,928	9	0,885
Mean	9,33	0,807	7,50	0,791	7,83	0,822	7,17	0,785

Mean of crossbred males (II ♂×III ♀ and III ♂×II ♀): 0,807 mg.
» » control » (I and IV): 0,797 mg.

TABLE 7. *Analysis of variance of experiment 3.*

Source of variance	Degrees of freedom	Mean square	Variance ratio
Between lines	3	0,08930	
Crossbred versus control males	1	0,03331	3,05
Between reciprocally crossbred males	1	0,15453	14,14***
Between control lines	1	0,08007	7,33**
Between generations	5	1,97901	181,1***
Line-generation interaction	15	0,00833	—
Between groups of males of common paternity within lines and generation	167	0,01093	2,48***
Between males of common paternity	1163	0,00441	
Total	1353		

** P 0,01 — 0,001. *** P < 0,001.

to form the initial population for lines I and II; *Tunnelgatan, Örebro* and *Florida* were used for lines III and IV. The selection value of the tested males in this experiment was judged from the body weight of as well male as female offspring, but as the weight of the females may depend on egg deposition and thus are less reliable as a measure of inherited body development, only the male values were included in the

calculations. Because of the more laborious testing procedure a maximum of 11 males per line were tested in each selection cycle, and of these the best three were selected for propagation.

In Fig. 4 and Tables 6 and 7 the results of experiment 3 are shown. The outcrosses of the foundation stocks show no increase in body weight, which thus seems to be little influenced by hybrid vigour. There is no apparent effect of selection during the 6 cycles; instead, most of the generations have values below those of the initial populations. Probably this failure of selection is due to uncontrollable environmental differences between vials, in spite of the efforts to standardize the culture conditions. As each group of common paternity is reared in a separate vial this environmental variation cannot be separated from hereditary differences between these groups. The experimental method was therefore inappropriate for body weight selection, and the experiment was broken off after 6 cycles. It has been included in this report only because the prime comparison, the one between crossbred and control offspring, points in the same direction as in experiments 1 and 2, *viz.* towards a better performance of the crossbred animals. The difference, 0,01 mg or 1,25 %, is however insignificant also in this case (5 % ratio: 3,90). Incidentally a significant difference between reciprocal crosses may be noted, revealing a maternal influence on body weight.

IV. DISCUSSION

The characters studied in the experiments, egg production, hatchability and body weight, represent different physiological conditions, presumably guided by different genetical systems. The effect of heterosis is great what concerns egg production and hatchability but inconsiderable for body weight. Also the susceptibility to environmental conditions varies. Hatchability is less influenced, egg production shows great seasonal fluctuations, and for body weight the inter-vial differences are great enough to mask the hereditary differences between groups. None of the experiments show a clear-cut and lasting effect of selection beyond the level of the initial populations. Three reasons may be given for this failure. Firstly, the initial populations already maintain a high level of performance because of their hybrid vigour. Secondly, low heritability and environmental fluctuations may reduce the genetical gain. Thirdly, the number of tested males, the selection differential and the number of selection cycles may be to small to give appreciable effects within the duration of the experiments.

The principal aim of the investigation, to test the value of the RRS method, is however not absolutely confined to improvements by selection. As all tests were made simultaneously for crossbred and control offspring, each cycle of selection gives a reliable comparison between the breeding methods, independent of the general level of performance. All three experiments point in the same direction, which is in favour of the RRS method.

In the *Drosophila* experiments by BELL, MOORE and WARREN (1954), egg production and egg size were studied. Their results agree with those of the present investigation in showing a small advantage of the RRS method over closed flock selection. Individual merit was made use of in the latter selection, and therefore the increase was more rapid in the closed flock but was later overtaken by the RRS line. The maximum performance was, however, not reached by any selection method but in some of the crosses performed between inbred lines.

The primary question to be asked in consequence of the results seems to be: why is the advantage of the RRS method so small? Theoretically it should be advantageous to other systems whenever overdominance is present. In case of complete or incomplete dominance the RRS method should be superior unless the initial population for closed flock selection contains as many favourable alleles as the two initial populations for RRS taken together. If epistatic interactions are of considerable importance, no general results can be predicted. A special case of interaction is where the added contribution of each favourable gene becomes less the higher the genetically determined level of performance is. This kind of interaction will function as a ceiling which cannot be surpassed by means of selection.

The small advantage of the RRS method may thus depend on: (1) scarcity of loci with overdominance, (2) limited number of favourable allels and (3) epistatic interactions.

The demonstrations of single gene overdominance in *Drosophila* are few. STERN *et al.* (1952) investigated 77 sex-linked lethals and found a few cases where the lethal gene in heterozygous condition raised the viability. HEXTER (1955) investigated six recessive mutants without finding any evidence of such a mechanism. Some other alleged cases may be explained without assuming overdominance. Thus it seems as if the phenomenon is rare, and where it is present its effects are not very striking.

This fact alone may be sufficient to answer the question of the small advantage of the RRS method. It does, however, not explain why there

is no real improving effect of selection over the level of the initial crossbred populations or why BELL, MOORE and WARREN found the highest performance in crosses between inbred lines. The second of the points mentioned above may restrict the effects of selection, but it can hardly be prevailing in the present case. The foundation stocks were all unrelated, and as judged by the heterosis in their crosses, also genetically divergent. It is unlikely that the two initial populations formed by the crosses should contain exactly the same alleles, so that further crosses between them would not increase the total number of favourable alleles.

The third point remains as a possible explanation. Epistatic interactions must be an important mechanism for the determination of specific and general combining ability which are well known from crosses between inbred lines. They may also be responsible for the formation of genetically conditioned ceilings and thus explain failures of selection improvements. Unfortunately, their effects are impossible to predict from case to case, and any breeding method that rests on such assumptions must always be tested with the actual stocks to be used in practice. From the results of the present investigation this is the primary conclusion to be drawn. The second, and more promising one, is that even under such circumstances the RRS method seems to give a small but general advantage.

SUMMARY

(1) Three experiments were undertaken with *Drosophila melanogaster* in order to test the value of the reciprocal recurrent selection (RRS) as a breeding method. It was compared to equally designed closed flock selection from the same initial population. Selection was entirely based on progeny tests of males; females were not tested, and individual merit was not considered. As selection criterion egg production was chosen in exp. 1, hatchability of eggs in exp. 2, and body weight in exp. 3. The experiments were continued for 20, 13, and 6 cycles of selection, respectively.

(2) The initial populations were made up by crossing together different stocks, and thus already showed hybrid vigour. Selection did not lastingly improve the level of performance of the initial populations.

(3) Taken over the whole experiments, the crossbred animals produced by the RRS method were superior to the purebred ones of the control lines in all three experiments. In exp. 1 the advantage was 6 %

and clearly significant, in experiments 2 and 3 the advantage was less than 2 % and not significant.

(4) The results are interpreted as due to scarcity of loci showing overdominance with striking effect and presence of epistatic interactions.

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THE CYTOLOGICAL BEHAVIOUR OF ACCESSORY CHROMOSOMES IN CENTAUREA SCABIOSA

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IN addition to the normal ten pairs of chromosomes in *Centaurea Scabiosa* there are certain plants possessing a number of extra chromosome elements (FRÖST, 1948). Such extra chromosomes, not homologous with those of the ordinary complement, have been given many different names. In a preliminary note (FRÖST, 1948) they were called B chromosomes, but now the term »accessory chromosomes» has been adopted in the order to have a more uniform name for this type of chromosome. The name »accessory chromosomes» was suggested by HÅKANSSON (1945) and MÜNTZING (1945), and later used by, *e. g.*, BOSEMARK (1950), MELANDER (1950), VAARAMA (1950), DOWRICK (1952), and VIRKKI (1954).

I. MITOSIS

1. Material and methods

The plants used for these cytological studies originated from natural populations of *Centaurea Scabiosa*, which have been mainly collected in Sweden and Denmark.

For chromosome counts root-tips were fixed in chrome-acetic-formalin (MÜNTZING's modification of NAVASHIN's fluid; MÜNTZING, 1933), embedded in paraffin, and sectioned at *ca.* 16 μ. The plants were kept at a temperature of *ca.* $+2^\circ$ for 12 hours before fixation. The sections were stained with crystal violet. Squashes have also been used to give better information of chromosome morphology and in this case the root-tips were pre-treated in oxiquinolin and stained with acetic-orcein following the method of TJIO and LEVAN (1952).

2. The normal chromosome complement

Among all the 20 ordinary chromosomes of *C. Scabiosa* one constriction is clearly distinguishable, but the position of this constriction

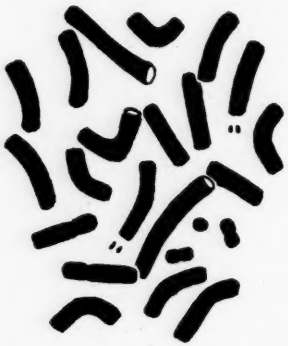


Fig. 1. Somatic metaphase from a plant with $2n=20+1$ standard accessory chromosome+2 accessory iso-chromosomes. — c:a 2800×.

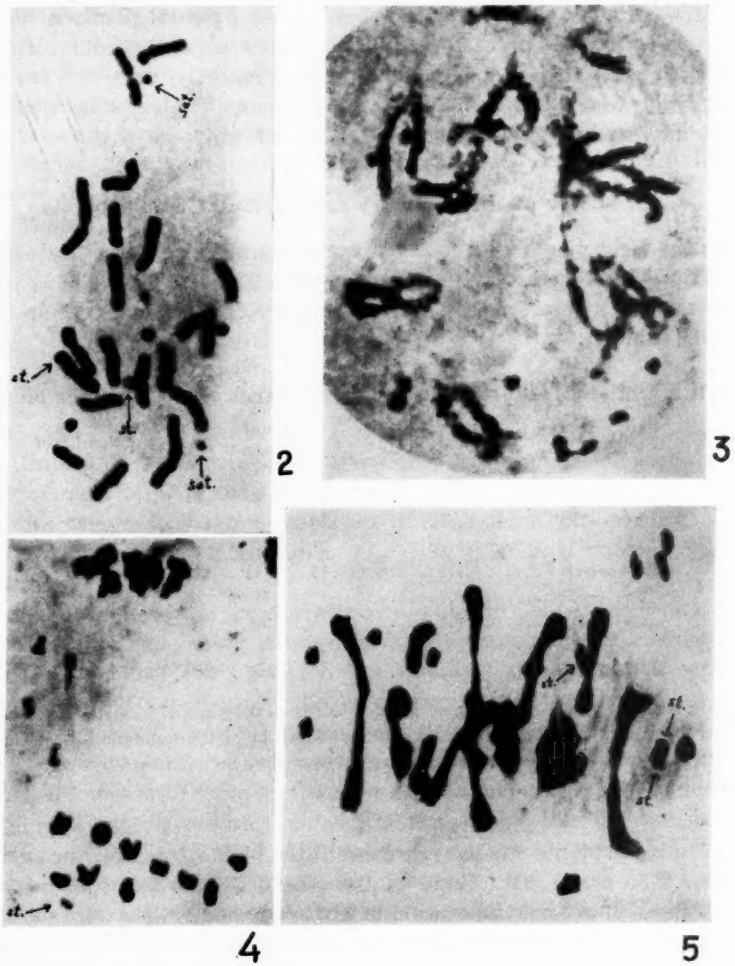
varied in the different chromosomes. However, it is usually submedian or median in position, but in some chromosomes it is sub-terminal. At least two of the chromosomes have satellites; these however, are definitely smaller than the accessory chromosomes (Figs. 1—2).

3. The accessory chromosomes

The accessory chromosomes differ mainly from the ordinary ones in their smaller size. Two types of different sizes have been observed.

The smallest type is the commonest one in natural populations and has therefore been called the standard type. These chromosomes generally look like small round bodies, but when more slender one can see that they are more or less rod-shaped. Their length is about one-fifth the length of the smallest of the ordinary chromosomes at somatic metaphase plates (Fig. 2). The meiotic studies indicate that they very probably have a terminal or sub-terminal centromere. These accessory chromosomes of the standard type have been found in plants from very different geographic regions, *e. g.*, Sweden, Yugoslavia, Austria, and England.

The other type of accessory chromosomes in *Centaurea Scabiosa* is about double the size of a standard type and has a median constriction (Fig. 1). It is probably an iso-chromosome derived through misdivision of the standard type. It has not been possible to prove this assumption by cytological observations, but it is supported by the fact that this metacentric chromosome is generally formed in low frequency in crossing experiments between plants with the standard type. The production of the iso-type is also more frequent when the number of ac-



Figs. 2—5. Photographs from aceto-orcein squashes of mitosis and meiosis in *Centaurea Scabiosa*. — Fig. 2. Somatic metaphase from a plant with $2n=20+5$ st. acc. chr. (oxiquinolin treatment). — Fig. 3. Diplotene from a plant with 10 st. acc. chr.; 4 of the accessories are associated with each other forming two bivalent-like configurations. — Fig. 4. Anaphase I in a plant with 1 st. acc. chr.+2 acc. iso-chr. The accessory iso-chromosome univalents have divided, while the univalent of the standard accessory chromosome is still undivided and has a polar position. — Fig. 5. Metaphase I in a plant with 12 accessories. —
Fig. 2, c:a 2200×; Figs. 3—5, c:a 2600×.

cessory chromosomes of standard type in the parental plants is high. The accessory iso-chromosome is also found in plants from natural populations of *Centaurea Scabiosa*. Its frequency is, however, low in proportion to the standard type. Most often one finds the iso-type in populations where the frequency of the standard type is high.

4. Constancy of the accessory chromosomes within plants

In the eight plants used for this investigation the roo-tip mitosis was examined in altogether 211 metaphase plates. The results obtained show that there is a small variation in the number of accessories within the plant (Table 1).

TABLE 1. *Analysis of the constancy of the accessories within the plant.*[1]

Plant No.	Number of accessories					Number of cells
	1	2	3	4	5	
K 85 b—362	1	35	4	1		41
» —391	1	28	1			30
» —119		31				31
K 14 a—44		17	(1)			18
» —38			18			18
» —39			28	2		30
» —48				20		20
K 85 b—109			2	20	1	23

[1] In every plant the chromosome number has been determined in metaphase plates from 3 to 4 root-tips. The data from different root-tips of the same plant have, however, been taken together as no evidence of variation between root-tips within the plant has been found.

The eight plants studied represent sister plants from two progenies (Nos. K/85 and K/14). Three of the plants (K/85—326, K/85 b—391, K/85 b—109) are not chosen completely by chance as they were selected from individuals which at the routine determination of the chromosome number (when only 3 to 4 cells per plant were studied) were found to have varying numbers of accessories. The other plants which were taken at random do not seem to have the same degree of variation. It is thus possible that the proportion of cells with deviating chromosome numbers is somewhat too high in proportion to the mean variation of accessory chromosomes in *C. Scabiosa*. However, it is clear that there does exist a definite but rather slight degree of variation. It is also possible that there is a difference betwen different plants in this respect. It must

be pointed out that it is easier to determine accurately the variation in number of accessories within a plant, when the number in the cell is above the »mean» value of accessories, rather than below. When the number of accessories established is below the »mean» value, there may be a risk that the missing accessories are hidden by the normal chromosomes. This is the reason why cells with accessory chromosomes above the mean value are more frequent than those below the value in Table 1.

Sometimes the impression is given that this variation increases when the number of accessories in the plant is high. This is, however, difficult to establish definitely for reasons pointed out above.

5. The position of the accessory chromosomes in somatic metaphase plates

Usually the smaller chromosomes have a more central position on the metaphase plate than the larger ones of a complement. Accessory chromosomes, which in general are smaller than the ordinary ones, should therefore in accordance with this rule have a more central position.

The position of the accessory chromosomes in *Centaurea Scabiosa* in the somatic metaphase plate has been investigated by the method described by MÜNTZING (1948): A line is drawn connecting the centromeres on the periphery of the metaphase plate, and these lines are drawn in such a way that the angle between them at both sides of the centromere is always at least 180°, as seen from the outside of the plate. Chromosomes with their centromeres on this line are called peripheral, while chromosomes with their centromeres inside this line are called central. With this method both types of accessory chromosomes (standard type and the iso-chromosome) have been investigated. It has in many cases not been possible to determine the position of the centromere. Instead, the periphery line has been drawn to the middle of the chromosome. The error introduced is probably very small, as the normal chromosomes have most often a median or sub-median centromere.

The material used for studying the accessory chromosomes of the standard type consisted of sister plants from a cross of the type (20+1 st. acc. chr.) × (20+1 st. acc. chr.), Table 2 *a*, whereas for the investigation of the iso-chromosome, plants have been used which originated from a natural population in northern Yugoslavia (Table 2 *b*).

The statistical analysis shows that both types of accessory chromo-

TABLE 2. *The position of the accessory chromosomes in somatic metaphase plates. P = peripheral chromosomes, C = central chromosomes.*

	Number of acc. chr	Normal chromosomes P	C	% C	Acc. chr. P	C	% C
a.	20+1 st. acc.	1364	1316	49,10	31	103	76,87
	20+2 » »	1451	1389	48,91	66	218	76,76
	20+3 » »	132	108	45,00	7	29	80,56
		2947	2813	48,84	104	350	77,09
b.	20+1 acc. iso.	294	306	51,00	5	25	83,33
	20+2 » »	264	276	51,11	12	42	77,77
		558	582	51,05	17	67	79,76

$\chi^2_a = 134{,}4$ $P < 0{,}001$ $\chi^2_b = 25{,}89$ $P < 0{,}001$

somes have a more central position in the somatic metaphase plates than the normal chromosomes.

II. MEIOSIS

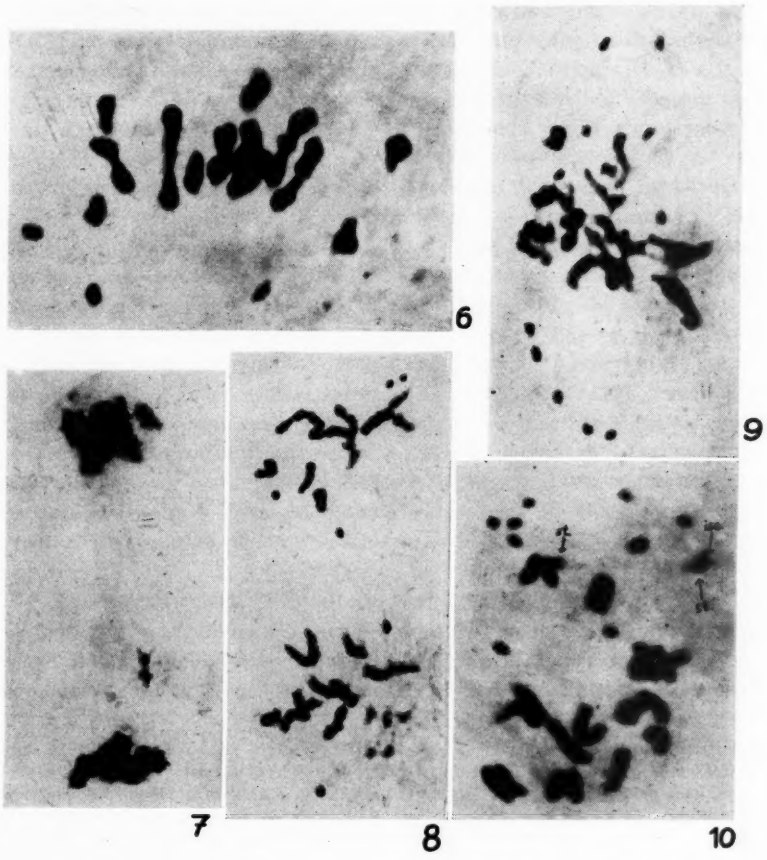
When studying meiosis, sections and squashes have been used. For sectioning, the buds were pre-treated in acetic-alcohol for 2 mins. before fixing in chrome-acetic-formalin, and stained with crystal violet; and, for squashing, the buds were fixed in acetic-alcohol and stained with orcein.

Centaurea Scabiosa is rather an unsatisfactory material for cytological studies at meiosis. The cytoplasm in the P.M.C. is also often stained; and rather frequently the fixations fail.

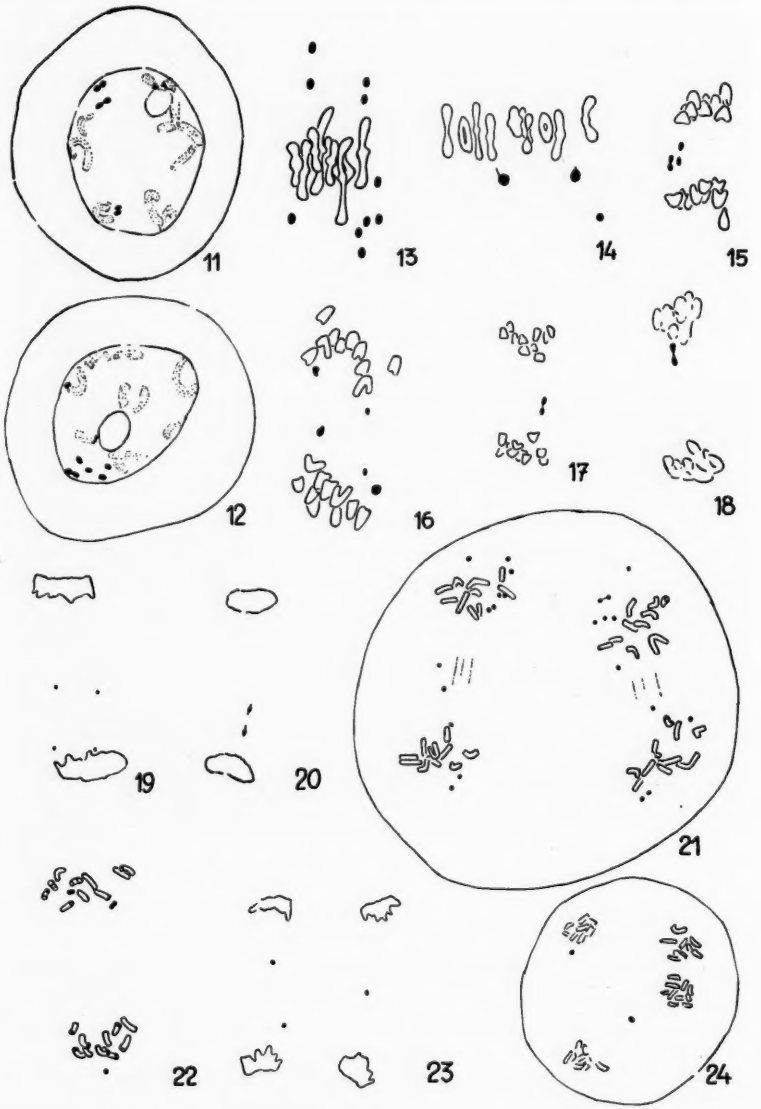
The ordinary chromosomes usually form ten bivalents during metaphase I, and of these 2 to 3 are ring- and 7 to 8 rod-bivalents. A higher number of ring bivalents might be expected in *Centaurea Scabiosa*, when most of the chromosomes have a median or submedian centromere (Fig. 2), but the low number of ring bivalents indicate a relatively low chiasma frequency. Rather often disturbances can be seen in the pairing behaviour between the normal chromosomes. In several cases univalent chromosomes have been noticed; asyndetic plants have been observed twice. Several cases of disturbed meiosis in *C. Scabiosa* have also been studied by CZAPIK (1954).

1. The accessory chromosomes of standard type

The meiotic behaviour of the accessory chromosomes in *C. Scabiosa* agree with those found in other species in not showing any pairing with the ordinary chromosomes.



Figs. 6—10. Photographs from aceto-orcein squashes of meiosis in *Centaurea Scabiosa*. — Fig. 6. Metaphase I in a plant with 1 st. acc. chr.+2 acc. iso-chr. — Fig. 7. Late anaphase I in a plant with 1 acc. iso-chr. The univalent iso-chromosome is still undivided. — Fig. 8. Anaphase II in a plant with 12 accessories (this photograph demonstrates the right anaphase group of the drawing Fig. 21). The distribution of the accessories at anaphase II seems to be more or less at random. — Fig. 9. Metaphase II in a plant with 12 accessories; 11 accessories are visible in the figure. — Fig. 10. Metaphase II in a plant with 10 st. accessories and 2 accessory isochromosomes (all of which seems to have divided at anaphase I, thus appearing as daughter univalents in this figure). — Figs. 6—10, c:a 2600×.



Figs. 11—24. Meiosis in various plants with accessory chromosomes. — Figs. 11—12. Two diplotene celles in a plant with 6 st. acc. chr. Some of the accessories show some kind of association. — Fig. 13. Metaphase I in a plant with 10 st. acc. chr. —

It has been possible to study the earlier stages of meiosis in some cases and during diplotene and diakinesis a low frequency of the accessory chromosomes seems to be associated with each other. An exact determination of the frequency of such associations has not been possible. Only in a plant with six accessory chromosomes could a more careful study be made. During diplotene in 36 cells an average of 18 per cent of the accessories were apparently associated with each other in bivalent-like configurations (Fig. 3 and Figs. 11—12). It is unlikely that the chromosomes in these cases are associated by chiasmata, for it has not been possible to detect with certainty any bivalents of the accessory chromosomes during metaphase I. It is more probable that these associations represent a general attraction or some degree of stickiness.

The accessory chromosomes of several plants are known to be heterochromatic, *e. g.*, *Zea*, *Anthoxanthum*, and *Festuca*. In the root-tips of *Centaurea* it has been impossible to decide whether the accessories are heterochromatic or not. However, during diplotene and earlier stages clear differences in stainability between the accessories and the ordinary chromosomes have been seen. The accessories in these cases show positive heteropycnosis; these differences in stainability do not always appear.

It has already been pointed out that no definite cases of bivalents formed by the accessory chromosomes have been seen in *Centaurea Scabiosa* during metaphase I. In a few cases, however, configurations suggesting some kind of association have been seen. The accessory chro-

Fig. 14. Metaphase I in a plant with 1 st. acc. chr.+2 acc. iso-chr. — Fig. 15. Anaphase I in a plant with 2 st. acc. chr. in different stages of division or preparation for division. — Fig. 16. Anaphase I in a plant with 1 st. acc. chr.+2 acc. iso-chr. One of the univalent accessory iso-chromosomes and the standard accessory chromosome have divided. One of the Iso-chromosomes is still undivided and situated at the polar group. — Fig. 17. Anaphase I in a plant with 1 acc. iso-chr. The univalent seems to prepare for division. — Fig. 18. Late anaphase I in a plant with 1 st. acc. chr. The univalent is still undivided and situated close to one of the polar groups. — Fig. 19. Early telophase in a plant with 2 st. acc. chr. Daughter univalents from the accessory chromosomes are seen to lag. — Fig. 20. Lagging accessory univalent or daughter univalents at late anaphase I (or early telophase I) in a plant with 1 acc. iso-chr. — Fig. 21. Anaphase II in a plant with $2n=20+12$ st. accessories.; 12 accessories are visible in the left anaphase, 7 of them are moving to one pole and 3 to the other, 2 are lagging. In the right anaphase, where 11 accessories are visible (the same anaphase as in Fig. 8), the distribution of the accessories to respective poles is 7 to 4. — Fig. 22. Anaphase II in a plant with 1 st. acc. chr.+2 acc. iso-chr. of daughter univalent type. The iso-chromosomes have moved to one pole and the standard accessory chromosome to the other. — Figs. 23—24. Late anaphase II showing lagging daughter univalents from the accessories. — Figs. 11—13, 15, 18, 19, c:a 1600×; Figs. 14, 16, 20—22, c:a 1300×; Figs. 17, 23—24, c:a 1100×.

TABLE 3. *The position of the univalent accessory chromosomes in relation to the equatorial region at metaphase I.*

Plant No.	Number of acc. chr.	% in the equat. region	% outside the equat. region	Number of cells
S—60—7	1	51	49	80
S—60—12	1	29	71	33
47 b—10	1	57	43	79
47 a—74	1	53	47	32
Total in plants with 1 acc. chr.	1	49	51	224
7—21	2	50	50	41
S—60—5	2	43	57	52
S—39—2	3	42	58	77
S—53—12	6	49	51	56
Total in plants with 2, 3 and 6 acc. chr.		46	54	226
Total in all the plants		47	53	450

mosomes were in these cases united with a rather long and very thin chromatin-thread. Nevertheless, it is improbable that these configurations represent true bivalents; they may merely be caused by some kind of stickiness.

The position of the univalent accessory chromosomes in relation to the equatorial region at metaphase I has been determined in 8 plants (Table 3). The region which is occupied by the bivalents of the ordinary chromosomes is considered the equatorial region. The lines which delimit the equatorial region have thus been drawn approximately through the centromeres of the normal bivalents (*cf.* LIMA-DE-FARIA, 1955).

As seen from Table 3, on an average 47 per cent of the univalent accessory chromosomes localize themselves in the equatorial region. In this respect they have about the same ability as univalent accessory chromosomes in *Festuca pratensis* (BOSEMARK, 1954). It has also been of interest during these studies to see whether there are any differences between plants with only one accessory chromosome and plants with two or more. No difference in position could be demonstrated. Thus, it seems that the position of the univalent accessory chromosomes during metaphase I is independent of their number.

The univalent accessory chromosomes generally divide during anaphase I. The frequency of lagging chromosomes (univalents and daugh-

TABLE 4. *Lagging accessory chromosomes at late anaphase I.*

Plant No.	Number of acc. chr.	% at one of the poles	% between poles	Number of cells
S—60—12	1	80	20	25
47 b—10	1	75	25	35
29—352	1	87	13	128
7—21	2	83	17	41
S—39—2	3	(96)	(4)	(15)
Total		86	14	244

ter univalents) during late anaphase I has been determined in six plants (Table 4).

In these plants the frequency of lagging chromosomes is relatively high, but it is not certain that all these laggards will be eliminated. Micro-nuclei have been observed in several cases during telophase I, and it is possible that a certain frequency of the accessories will be eliminated during the first division.

The accessory chromosomes do not always divide during anaphase I. In certain cases during late anaphase I and telophase I figures are found showing that the daugheter chromatids have not been separated from each other (Figs. 7 and 18). It is possible in these cases that the accessory chromosome will be included undivided in the telophase-nuclei. An estimation of the frequency of still undivided accessory chromosomes during late anaphase I is given in the following values: In three plants with one accessory chromosome a total of 146 late anaphases I were studied; among these 22 cases were seen where the accessory chromosomes was still undivided, and often situated close to one of the poles.

The later stages of meiosis have only been studied in a few plants. During metaphase II the ability of the accessory chromosomes to localize themselves on the plate seems to be about the same as for the univalents during metaphase I (Figs. 9—10).

During late anaphase II the number of lagging accessories are, as during anaphase I, relatively great (Table 5 and Figs. 21—24).

TABLE 5. *Lagging accessory chromosomes at anaphase II.*

Plant	Number of acc. chr.	% at one of the poles	% between poles	Number of cells
S—60—12	1	72	28	53
47 b—10	1	64	44	27
7—21	2	78	22	18
S—39—2	3	82	18	87

Micro-nuclei have also been observed during telophase II, and judging by these it might be expected that a considerable number of the accessories are also eliminated during the second division.

2. The accessory iso-chromosome during meiosis

The behaviour of the accessory iso-chromosome during meiosis agrees fairly well with that of the standard type. During metaphase I they do not pair with the ordinary chromosomes, nor do they pair with each other (Figs. 6 and 14). The position of the univalent accessory iso-chromosomes during metaphase I has been determined in one plant with $2n=20+1$ st. acc. chr. $+2$ iso-acc. chr. (Table 6).

TABLE 6. *A comparison of the position of the two types of univalent accessory chromosomes at metaphase I in a plant with* $2n=20+1$ *st. acc. chr.* $+2$ *acc. iso-chr.*

	% in the equat. region	% outside the equat. region
The standard type	59	41
» iso-type	44	56

Number of cells=61. $\chi^2=3{,}52$. $0{,}10 > P > 0{,}05$.

The values obtained for the standard and the accessory iso-chromosomes do not show any significant difference.

Similar to the standard type the accessory iso-chromosome generally divides during anaphase I. In a plant (J—24—31) with only one accessory iso-chromosome 50 late anaphase I were studied: in 31 of these the accessory iso-chromosome had clearly divided. In 9 cases the iso-chromosome was still undivided; in these cases it was often situated rather close to one of the poles (Fig. 7). It is possible that in such cases the iso-chromosome will often be included undivided in the telophase nucleus. (In 10 cases it was not possible to decide whether the iso-chromosome had divided or not, but in these cases no lagging iso-chromosomes could be observed.)

Lagging accessory iso-chromosomes could often be seen during late anaphase I, and of the 50 late anaphases I studied in the plant (J--24—31), lagging accessories were seen in 20 cases (univalents and daughter univalents). (Figs. 7 and 20.)

Also during anaphase II lagging iso-chromosomes can be seen, and during telophase II micro-nuclei are visible.

III. DISCUSSION AND CONCLUSIONS

The most common of accessory chromosomes in *C. Scabiosa*, the standard type, is easily distinguished from the ordinary chromosomes owing to its very small size. In somatic mitosis (metaphases) it has often a more or less globular appearance. The standard type has been found in plants from very different regions of Europe and they always seem to be of the same morphological type.

The second type of accessory chromosomes found in *C. scabiosa* is about double the size of the standard type and has a median constriction. This type is but seldom found in natural populations, whereas, like the standard type, it has been found in plants from very different geographic regions. For reasons pointed out on page 416 it was assumed that this type is an iso-chromosome derived from the accessory standard chromosome. Accessory iso-chromosomes have been found in several plant species, *e. g.*, *Secale* (MÜNTZING, 1944), *Anthoxanthum* (ÖSTERGREN, 1947), *Chrysanthemum* (DOWRICK, 1952), and *Festuca* (BOSEMARK, 1956). Accessory iso-chromosomes seem to be rather frequent in some species of grasshoppers (WHITE, 1954). The relatively frequent production of iso-chromosomes from the accessories through misdivision of the centromere is probably caused by the fact that accessory chromosomes often are univalent during the meiotic division.

The constancy of the accessory chromosomes within plants was studied in root-tip mitosis. It was found that there was a small but obvious variation in the number of accessories within the plant. Such variation has been reported in *Zea mays* (DARLINGTON and UPCOTT, 1941) and *Godetia viminea* (HÅKANSSON, 1949). The accessory chromosomes in *Poa alpina* (MÜNTZING, 1948; MÜNTZING and NYGREN, 1955) and *Sorghum* (DARLINGTON and THOMAS, 1941) belong to a special category: for in these species they are generally eliminated in the root-tips and other somatic tissues, and thus are only present in the germ line, whereas in other species such as *Secale* (MÜNTZING, 1948), *Anthoxanthum* (ÖSTERGREN, 1947) and *Festuca* (BOSEMARK, 1954) the number of accessory chromosomes is remarkably constant within one individual.

The position of the accessory chromosomes in somatic metaphase plates has been determined following the method described by MÜNTZING (1948). It was found that the accessory chromosomes in *C. Scabiosa* (the standard type as well as the iso-chromosome) have a very strong tendency to occupy a central position in the somatic metaphase plates.

The same is the case in rye (MÜNTZING, 1948) and in *Festuca pratensis* (BOSEMARK, 1954) and from their investigations MÜNTZING and BOSEMARK conclude that the accessory chromosomes in these species have a centromere of normal strength. On the other hand, in maize the accessory chromosomes have a strong tendency to move to a more peripheral position in the somatic metaphase plates. DARLINGTON and UPCOTT (1941) considered this situatoin to be an indication that the accessory chromosomes in maize have a more or less defective centromere. The defective centromere may lead to non-disjunction and elimination of the accessories, a phenomenon which will result in a certain variation in their number within the plant. The conclusion to be drawn from the results in *Centaurea Scabiosa* is that the accessory chromosomes probably have a centromere of normal strength. This conclusion, however, may be in conflict with the variation in the number of accessories within the plant. This variation might, according to DARLINGTON and UPCOTT, be evidence of a weakness in the centromere. The somatic variation in *Centaurea Scabiosa* is rather small, and also the accessory chromosomes are very small. It is therefore possible that the small size of the accessories causes them to have a central position in the somatic metaphase plates, in spite of a somewhat defective centromere. On the other hand, the small variation in number of accessories within the plant may have other causes than a possible weakness of the centromere. A simple explanation is that it may result from some kind of stickiness of the accessories which may sometimes prevent the separation of the daughter chromosomes at the anaphase movements.

Investigation of meiosis in *Centaurea Scabiosa* has shown that a characteristic property of the accessory chromosomes is that there are always univalents during metaphase I. In this respect the accessory chromosomes in *C. Scabiosa* agree with the accessories in *Caltha palustris* (REESE, 1954). The reason for this weak pairing need not necessarily be due to their small size. The small »fragments» in *Tradescantia* (WHITAKER, 1936) are very frequently paired with each other. WHITE (1954) suggests that heterochromatic segments prevent the formation of chiasmata and this may be the reason for the lack of chiasmata in *Centaurea Scabiosa*, as it is very probable that heterochromatic segments occur in the accessory chromosomes. However, this factor is of very little importance in *Caltha palustris*, as the accessory chromosomes in this species (according to REESE, 1954) are euchromatic. It can also be assumed that the chiasma frequency of the ordinary chromosomes is correlated with that of the accessory ones. As has been pointed out

earlier in *Centaurea*, the great number of rod-bivalents among the ordinary chromosomes indicate that the frequency of chiasmata is low.

During the meiotic prophases the accessory chromosomes show a low degree of pairing. These associations are probably due to some kind of attraction or stickiness, and do not represent true bivalents as they always seem to be disassociated before metaphase I. Similar associations during the meiotic prophase (not representing true bivalents) are found in several species, *e. g.*, *Parthenium argentatum* (CATCHESIDE, 1950) and *Festuca pratensis* (BOSEMARK, 1954).

The accessory chromosomes in *Centaurea Scabiosa* divide most frequently during anaphase I, and thus agree in this respect with the univalent accessory chromosomes in *Secale*, *Trillium* (SPARROW *et al.*, 1952), *Chrysanthemum* (DOWRICK, 1952), *Zea*, and *Festuca*. In other species univalent accessory chromosomes generally pass undivided to the poles during anaphase I, *e. g.*, in *Anthoxanthum*, *Godetia*, *Poa*, *Parthenium* (CATCHESIDE, 1950), and *Caltha* (REESE, 1954).

As pointed out by ÖSTERGREN (1947), the ability of accessory chromosomes to pass undivided to the poles during anaphase I will reduce the risk of their meiotic elimination. However, in some special cases even divided chromosomes may be only slightly eliminated. BOSEMARK (1954) has, thus, shown in *Festuca pratensis* that univalent accessory chromosomes which are dividing during anaphase I are eliminated in a very low frequency. A similar situation seems to hold good for the accessories in *Trillium* (SPARROW *et al.*) and for a very small chromosome derivative in rye (LIMA-DE-FARIA, 1955). For the standard fragment in rye, on the other hand, the degree of meiotic elimination for univalents dividing during anaphase I is very high (MÜNTZING, 1945).

In *Centaurea Scabiosa*, however, a certain degree of elimination of the accessory chromosomes may be expected during the meiotic divisions, as indicated by a relatively high percentage of lagging accessory chromosomes during anaphase I and II, and also by the presence of micronuclei.

SUMMARY

(1) The cytology of accessory chromosomes in *Centaurea Scabiosa* was studied.

(2) Two types of different sizes have been found. (i) The smallest one, which has been called the standard type, is the most frequent type in natural populations. Its size is about one fifth the length of the smallest

of the ordinary chromosomes. (ii) The other type is about double the size of the standard type and has a median constriction. This type is assumed to be an iso-chromosome derived from the standard type.

(3) The accessory chromosome of standard type was found to show a slight variation in number within the plant.

(4) The accessory chromosomes (the standard type and the iso-type) have a more central position in somatic metaphase plates than the normal chromosomes.

(5) The accessory chromosomes do not pair with the ordinary chromosomes. They seem always to be univalents at metaphase I and in most cases these univalents divide at anaphase I.

(6) The observation of lagging accessories at anaphase I and II and micronuclei at telophase I and II indicates that a considerable proportion are eliminated during meiosis.

(7) The meiotic behaviour of the accessory iso-chromosome appears to be similar to that of the standard type.

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THE CHROMOSOME COMPLEMENT OF THE RABBIT

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PAINTER (1926) was the first to make a thorough analysis of the rabbit chromosomes. The chromosome number was found by him to be 44, which has been confirmed by later authors. (For references see MAKINO, 1951.)

The domestic rabbit is a favourable experimental animal in several respects, and the knowledge of its genetics is steadily on the increase (SAWIN, 1955). Therefore, the present author considers it important to have available a morphological study of the rabbit chromosomes.

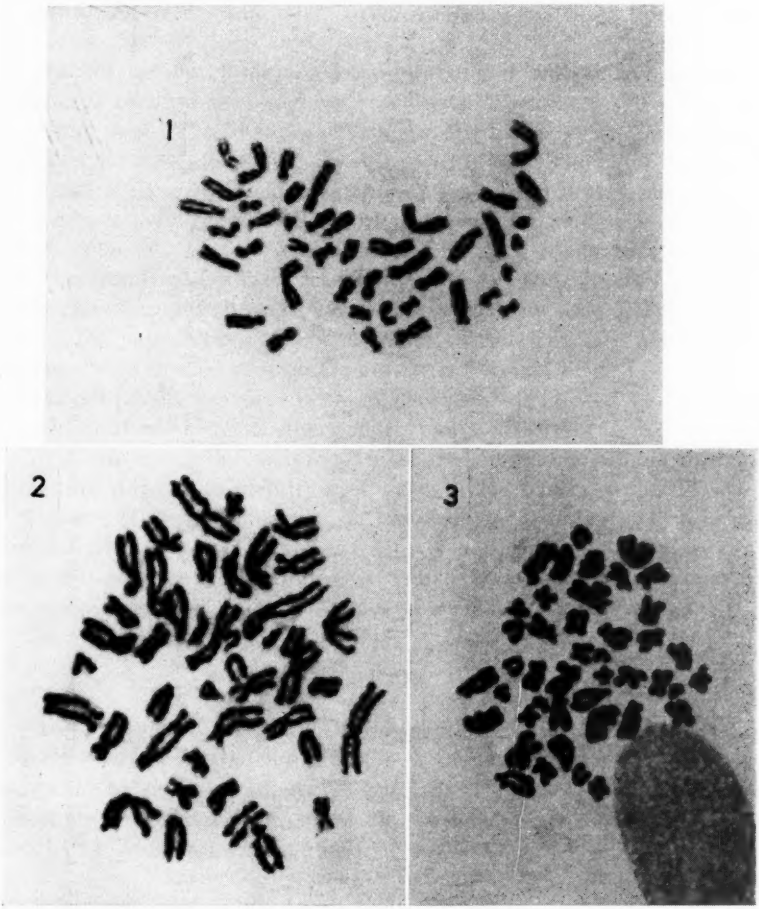
As it was not suitable to make a thorough morphological chromosome analysis from figures hitherto published, a technique to accentuate the position of the centromeres was used. This made it possible to obtain chromosome plates permitting an idiogram analysis.

MATERIAL AND METHODS

Swedish white country-bred rabbits were used. The chromosome preparations were acetic-orcein squashes of lung cells of 19-day-old embryos, the sex of which could easily be determined. As a basis of the idiogram analysis every single chromosome was drawn separately in the centre of the view field of the microscope at $\times 3700$, using a camera lucida.

When seriating the chromosomes of Fig. 4, chromosome size and the position of the centromeres were considered. The seriation could be performed with reasonable accuracy, as all the chromosomes of the four plates analysed were situated in one level, free from each other, and had, in all cases, clearly visible centromeric regions. This should always be a pre-requisite when making idiogram analyses.

If an organism has many small chromosomes, the picking out of the members of pairs among them becomes problematical owing to morphological similarities. Technical difficulties of accurate depicting will give rise to confusion. Consequently, the author does not pretend to have arranged every chromosome in its correct pair.



Figs. 1—3. The mitotic chromosomes of female (1) and male (2, 3) rabbit. — Photomicrographs, ×1090.

CHROMOSOME MORPHOLOGY

No regularly occurring secondary constrictions or other differentiations of the chromosome arms could be detected.

It was possible to arrange the chromosomes in a corresponding manner in all the plates of both the sexes. Two chromosomes of the male

Fig. 4. Four idiogram analyses of male (a, b) and female (c, d) rabbit.

could not be arranged in pairs. These are considered to be the sex chromosomes (Fig. 4).

Some of the chromosomes can be identified with a fair degree of certainty, because they deviate morphologically from the pairs surrounding them. The two largest pairs of chromosomes are metacentric, but it is not possible to distinguish between them. The pairs numbered 3, 4 and 5 show different locations of their centromeric regions and each one of them may be identified with some degree of certainty. The numbers 15, 18 and 21 have terminal centromeres or nearly so, and they may be identified by their relative length. The four smallest chromosome pairs, numbers 18—21, can be identified with some degree of certainty. The pair number 18 has a terminal centromere, 19 is metacentric, 20 has a subterminal centromere and 21 a terminal one.

It is not possible to identify the X chromosome, nor the Y. The latter may be confused with members of the pair number 20.

Thus, the conclusion may be drawn that individual rabbit chromosomes are difficult to identify even on quite clear mitotic plates.

The size of the chromosomes on Fig. 4 ranges from 7,0 to 1,7 μ in the pre-metaphase (a) and from 4,0 to 1,0 μ in plate (d) with the most contracted C-metaphase chromosomes. Thus, the largest chromosomes of the complement are about four times as long as the smallest ones.

SUMMARY

The 44 chromosomes of the domestic rabbit are pictured with a view to their relative size and the situation of the centromeres. The chromosomes can be arranged in pairs except for a metacentric X and a Y with subterminal centromere in the male. In most cases it is difficult to identify individual chromosomes.

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REFLEXED AND ERECT INVOLUCRE IN LACTUCA

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INTRODUCTION

THE garden lettuce, *Lactuca sativa*, L. is a species consisting of a large number of varieties which show great diversity in morphological and physiological characters. They fall into four quite well defined groups namely cabbage lettuce, cos lettuce, leaf lettuce and asparagus lettuce. With the exception of some colchicine induced autotetraploids all forms of cultivated lettuce are diploid with $2n=18$, and they cross easily with each other giving fully fertile hybrids.

THOMPSON, WHITAKER and KOSAR (1941) established a compatibility group within *Lactuca* which they call the subgenus *Serriola*. This consists of five species: *L. serriola*, L., *sativa*, L., *altaica*, FISCH. and MEY., *virosa* L., and *saligna* L., all diploid species with $2n=18$. The authors concluded that *L. serriola*, *sativa* and *altaica* should be considered as varieties of the same species as they cross readily in both directions and have many morphological characters in common.

It is not the intention to discuss in detail in this paper the taxonomy of the different species of the *serriola*-group but only to deal with one character which separates *L. sativa* and *L. altaica* from *L. serriola*, *L. virosa* and *L. saligna*. This character to which no reference has been found in any literature on *Lactuca*, concerns the behaviour of the involucral bracts at maturity. In *L. serriola* as in *L. saligna* and *L. virosa* these bracts become reflexed on the peduncle as soon as the fruits are ripe so that the pappus-carrying achenes are exposed and their distribution by means of the wind is facilitated. In all cultivated forms of lettuce and in *L. altaica*, however, the involucral bracts remain erect or spread horizontally, but are not reflexed on the stalk of the infructescence. The ripe achenes therefore usually remain enclosed in the fruiting head until this falls to the ground and although equipped with pappus they are not easily distributed by the wind. The difference between the two types of heads is illustrated by Figs. 1—2.

Fruiting heads of some *Lactuca* species are illustrated in HEGI's

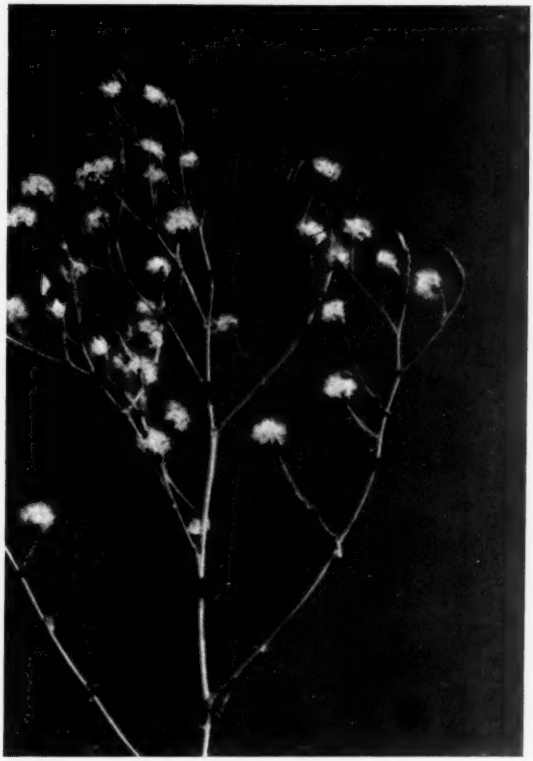

Fig. 1. *Lactuca sativa* (erect involucre).

»*Illustrierte Flora von Mitteleuropa*» although no reference to involucral characters is made in the text. *L. sativa* is illustrated with a typical erect involucre, *L. virosa* with one fruiting head showing the reflexed involucre and another still erect, *L. saligna* with the involucral bracts in the process of turning back. Only one fruiting head is illustrated for *L. serriola* and this shows the erect involucre. This is most probably an illustration of a young fruiting head, because the reflexed involucre is definitely normal in *L. serriola*. Six lines of this species from different sources have been in cultivation for many generations and have produced only plants with the typical reflexed involucre. In addition, plants have been raised from 13 different seed samples received from Botanical Gardens or collected from wild specimens and they have not yielded a

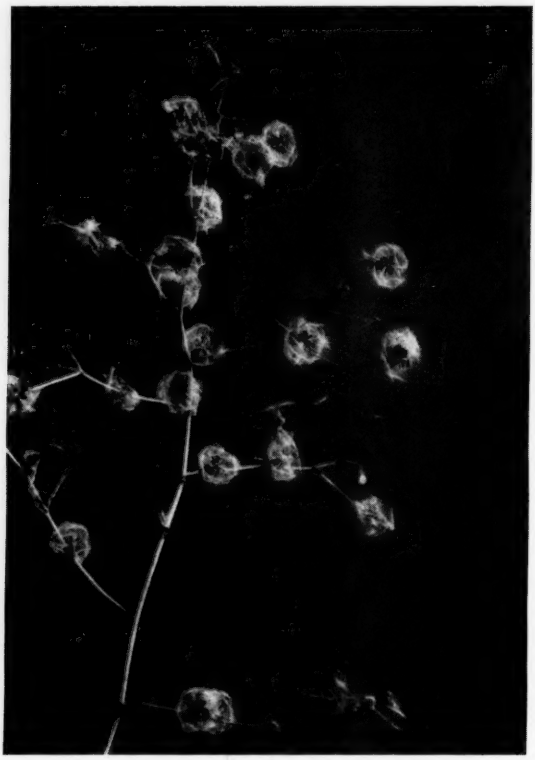

Fig. 2. *Lactuca serriola* (reflexed involucre).

single plant with erect involucres nor has it been possible to find such plants in numerous wild populations examined in England, Sweden, Belgium, and France.

L. altaica is not the only wild lettuce with erect involucres. This character is also found in certain wild forms which have yet to be identified satisfactorily. Material of these forms has been obtained as seed samples from various botanial gardens under the names of *L. serriola*, *virosa*, *saligna*, *sagittata*, *augustana*, REICHB. and *dregeana* D. C. Without going into taxonomical details it may be stated here that these forms constitute a fairly homogeneous group which is distinct from other forms of lettuce that have been under the writer's observation. They have 18 somatic chromosomes and give fertile hybrids with *L. ser-*

riola, altaica and *sativa*, but sterile hybrids with *L. virosa* and *L. saligna*. In many respects they agree with ALLIONI's description of *Lactuca augustana* in »*Flora Pedemontana*» but until their identity is definitely established it is preferable to let them remain unnamed. Two lines from this material have been used in crossings to be reported here and they have been designated »SL» and »SP».

CROSSES

Material and Methods

The inheritance of the involucral character has been studied in 31 crosses. A list of the parent lines and information about their origin is found in Table 1.

The method employed in crossing what that described by ERNST-

TABLE 1. *Parent lines.*

Abbreviation	Full name	Involucre	Origin
Asp.	Asparagus lettuce	Erect	Commercial Variety
Oakl.	Oakleaved; leaf lettuce	»	» »
Paris	Paris White Cos; cos lettuce	»	» »
R. rouge	Romaine rouge d'hiver de Vendée; cos lettuce	»	» »
Berl.	Berliner; cabbage lettuce	»	» »
K. driv	Kejsar driv (syn. Emperor forcing); cabbage lettuce	»	» »
wwyy	—	»	From: K.driv×Laurenzianer (cabbage lettuce)
Altaica	*L. altaica* FISCH. and MEY.	»	Royal Botanical Gardens, Kew, London, England.
SL	» *augustana* ALL.?	»	Botanical Garden, Lund, Sweden
SP	» » »	»	» » , Palermo, Italy
Sc. H	» *serriola* L.; entire-leaved	Reflexed	Wild population at Harwich, Essex, England
Sc. L.	» » »; lobed-leaved	»	Wild population at Lund, Sweden
Sc. R.	» » »; »	»	» » » Romford, England
Sc. S. Afr.	» » »; »	»	Wild population South Africa (loc. unknown)
Fl. 23	—	»	From: Rudolphs Liebling (cabbage lettuce) ×*L. serriola*

SCHWARZENBACH (1936), *i. e.*, emasculation before the pistils have grown through the anther tubes followed by washing with water in order to remove any pollen that may have adhered to the stigmata.

Autogamy is predominant both in wild and in cultivated lettuces but cross-pollination by insects occurs occasionally. Seed for progeny testing was therefore taken from branches isolated in vegetable parchment sleeves.

A serious handicap in the genetical work has been the fact that most of the material was very susceptible to fungus diseases, the most devastating of which is caused by *Botrytis cinerea*. This mould nearly always attacks lettuce plants at the fruiting stage in England and in wet seasons the result is often complete seed failure. Under such conditions it is impossible to make observations on involucral characters. It was therefore necessary to raise the seeds in a heated greenhouse during the winter and to transplant to the field as early as possible so that the plants reached the fruiting stage in July or August when conditions usually are dry enough to allow a reasonable development of the fruiting heads. As the greenhouse facilities have been very limited it has not been possible to raise any F_3 material in this way. Consequently no F_3 data are available for the involucral characters although several F_3 generations have been grown from crosses involving these characters.

Results

The reflexed involucre was found to be dominant in F_1, but the mechanism causing reflexion of the bracts appears to fail more often in heterozygotes than in homozygotes.

There was a clear cut segregation of the two parent types in F_2. The data are presented in Table 2. Results from crosses of the type erect × reflexed and reflexed × erect have been totalled separately. Both totals deviate somewhat from 3 : 1 and the deviation is almost significant for crosses of the type erect × reflexed. There is quite good agreement between crosses of the same type as shown by the heterogeneity χ^2s. The deviations follow opposite directions in the two totals and in consequence a very good fit for 3 : 1 is obtained when results from all segregating crosses are totalled. The figures are 3791 : 1226 with a χ^2 for deviation of only 0,8484. But the χ^2 for heterogeneity between the two types of crosses is 7,0045 which with only one degree of freedom corresponds to a P-value of less than 0,01. The discrepancy is due to a deficiency of F_2 plants with the involucral character of the mother parent

TABLE 2. *F_2-results.*

erect × erect				erect × reflexed					reflexed × erect				
Cross	Involucre		Total	Cross	Involucre		Total	χ^2 for 3 : 1	Cross	Involucre		Total	χ^2 for 3 : 1
	reflexed	erect			reflexed	erect				reflexed	erect		
Berl. × K. driv	0	199	199	Asp. × Fl. 23	220	78	298	0,219	Fl. 23 × R. rouge	178	63	241	0,167
Berl. × Asp.	0	280	280	Berl. × Fl. 23	74	17	91	1,983	Fl. 23 × Altaica	201	69	270	0,044
Berl. × Altaica	0	180	180	Berl. × Sc. H	346	107	453	0,460	Sc. L × Altaica	358	133	491	1,141
Berl. × SP	0	259	259	Berl. × Sc. L	202	60	262	0,616	Sc. S. Afr. × Altaica	179	75	254	2,777
K. driv × Alt.	0	168	168	Berl. × Sc. S. Afr.	338	108	446	0,146	Sc. L × SP	199	70	269	0,150
K. driv × SL	0	265	265	Berl. × Sc. R	118	52	170	2,831	Total	1115	410	1525	4,279
Asp. × Alt.	0	202	202	K. driv × Fl. 23	217	57	274	2,574	χ^2 for deviation from 3 : 1 : 2,891				
SL × Alt.	0	275	275	K. driv × Sc. H	47	22	69	1,744	χ^2 for heterogeneity : 1,388				
Paris × Alt.	0	259	259	Oakl. × Fl. 23	226	69	295	0,408					
Oakl. × Sp.	0	136	136	wwyy × Sc. H	351	101	452	1,699					
				Paris × Sc. L	206	59	265	1,058					
				Altaica × Sc. H	126	41	167	0,018					
				SL × Sc. L	205	45	250	6,533					
				Total	2676	816	3492	20,244					
				χ^2 for deviation from 3 : 1 : 4,961 [1]									
				χ^2 for heterogeneity : 15,282									

reflexed × reflexed			
Cross	Involucre		Total
	reflexed	erect	
Sc. S. Afr. × Sc. H	511	0	511
Sc. H. × Sc. L	60	0	60
Sc H × Sc. R	106	0	106

[1] $0{,}05 > P > 0{,}02$.

whether this character is the dominant or recessive. No explanation can be offered for the phenomenon, but this does not invalidate the conclusion that the erect involucre is inherited as a simple recessive character.

All crosses of the types erect×erect and reflexed×reflexed bred true in F_1 and F_2 (see Table 2) which proves that the recessive gene for erect involucre is the same in *L. sativa*, *L. altaica* and the unnamed lines »SL» and »SP». This recessive gene will be called *er*.

The reflexed involucre of *L. virosa* and *L. saligna* was found to be dominant in crosses with erect involucre forms, but sterility of the F_1 plants made it impossible to study further generations.

The possession of an inefficient mechanism for seed distribution must be a disadvantage under conditions of natural selection. The forms of wild lettuce with erect involucres appear to have a much more limited distribution than the forms possessing reflexed involucres and one reason for this is probably the effect of the *er* gene on the dispersal of the seed.

In cultivation, however, a character which increases the capacity of the plant to retain its seeds must be expected to have a selective advantage (*cf.* DARLINGTON, 1956, p. 127) even in a crop which is grown for the vegetative parts. It is therefore possible that the recessive character has arisen by mutation during cultivation and that it has gradually spread to all cultivated forms. But it is equally possible that the cultivation of lettuce started with wild forms which already had the erect involucre although not necessarily because of this character. This question will be discussed on a later occasion in the light of further data from crosses between species of the *serriola* group.

SUMMARY

In some forms of *Lactuca* the involucre is reflexed on the stalk of the fruiting head, exposing the achenes to the wind, in other forms, including all cultivated varieties, it remains erect, enclosing the fruits. A recessive gene, *er*, is responsible for the erect involucre.

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CYTOGENETICS OF ACCESSORY CHROMOSOMES IN PHLEUM PHLEOIDES

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I. INTRODUCTION

PHLEUM phleoides (L.) KARST. is a perennial grass species, widespread in Europe on dry, sandy and chalky soils, and extending to N. Asia and N. W. Africa (HUBBARD, 1954). The chromosome number has been determined to $2n=14$ by, among others, AVDULOV (1931), SOKOLOVSKAJA and STRELKOVA (1940) and BÖCHER (1950). Besides the diploid number, BÖCHER found the tetraploid number ($2n=28$) in two samples from the Botanical Gardens in Nancy, France and Erevan, Armenia, respectively. BÖCHER, in his study of chromosome behaviour and syncyte formation in *Phleum phleoides*, also describes the behaviour of accessory chromosomes found in five of the diploid and one of the tetraploid samples.

The main purpose of the present investigation has been to obtain information on the frequency of plants with accessory chromosomes in Swedish populations of *Phleum phleoides*, but, in addition, the cytology and transmission of the accessory chromosomes has been studied.

II. MATERIALS AND METHODS

In sampling the populations, whole plants were dug up at random and sent to the Institute of Genetics, Lund. The average sample size was 15—20 plants. At each locality notes were made about population size and density, soil type and humidity. After fixation of root-tips all plants were transferred to the experimental field.

In order to gain some idea of the reliability of the method of sampling, ripe spikes from about 40 plants were collected in one locality at the same time as the population plants. After thrashing and mixing the seed a sample of 100 seeds was taken out and sown, the chromosome numbers being determined in root-tips from the seedlings obtained. A comparison between the chromosome number distribution thus obtained and the chromosome numbers found in the established population plants for the locality is given in Table 1.

TABLE 1. *Comparison between the chromosome number distributions obtained for a population when applying two different methods of sampling.*

Method of sampling	Number of plants with respective numbers of accessory chromosomes					Total number of plants	% plants with acc. chr.	M. acc. chr. per plant
	0	1	2	3	4			
Established pop. plants	13	2	1	1	—	17	23,5	0,41
Seedlings from 40 pop. plants	39	12	4	1	1	57	31,6	0,47

As seen the agreement between the results obtained by the two methods is fairly good. This supports the author's opinion that a random sample of 15—20 plants from a limited population is sufficient to give a fairly good idea of the frequency of plants with accessory chromosomes in the population.

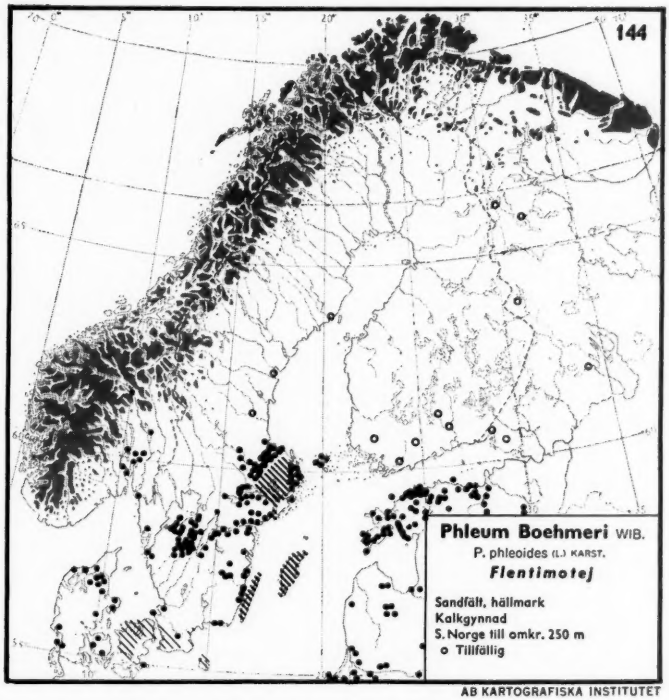
All chromosome counts were made in root-tips fixed in chrome-acetic formalin, sectioned at 14 μ and stained in crystal violet. Meiosis was studied mainly in aceto-carmine and Feulgen squashes after fixing in acetic-alcohol (1 : 3). Partly also sectioned material fixed in chrome-acetic formalin and stained according to the Feulgen formula was used. The first pollen mitosis was studied in aceto-carmine squashes after fixing in acetic-alcohol.

In studying the transmission of the accessory chromosomes, pair crosses were made by isolating together two plants in special isolation cages in the green-house.

III. FREQUENCY AND GEOGRAPHICAL DISTRIBUTION OF PLANTS WITH ACCESSORY CHROMOSOMES

The range of distribution of the species in Sweden is seen on Map 1. The distribution is discontinuous, and five main areas of distribution can be recognized, *viz.* (1) the Province of Skåne, (2) the »Falan» tract in the Province of Västergötland, (3) the Province of Uppland and surrounding areas, (4) the island of Öland and (5) the island of Gotland. The distribution is fairly typical of East-European continental calciphilous species in Sweden. Although the ecologic conditions vary somewhat between the areas, certain main features are common to all. Thus all five areas belong to the most arid districts in Sweden. The content of lime in the surface soil is also generally high, except in parts of the island of Öland.

Out of 1406 population plants chromosome-determined, 1402 were diploid with fourteen normal chromosomes. Two plants were found to

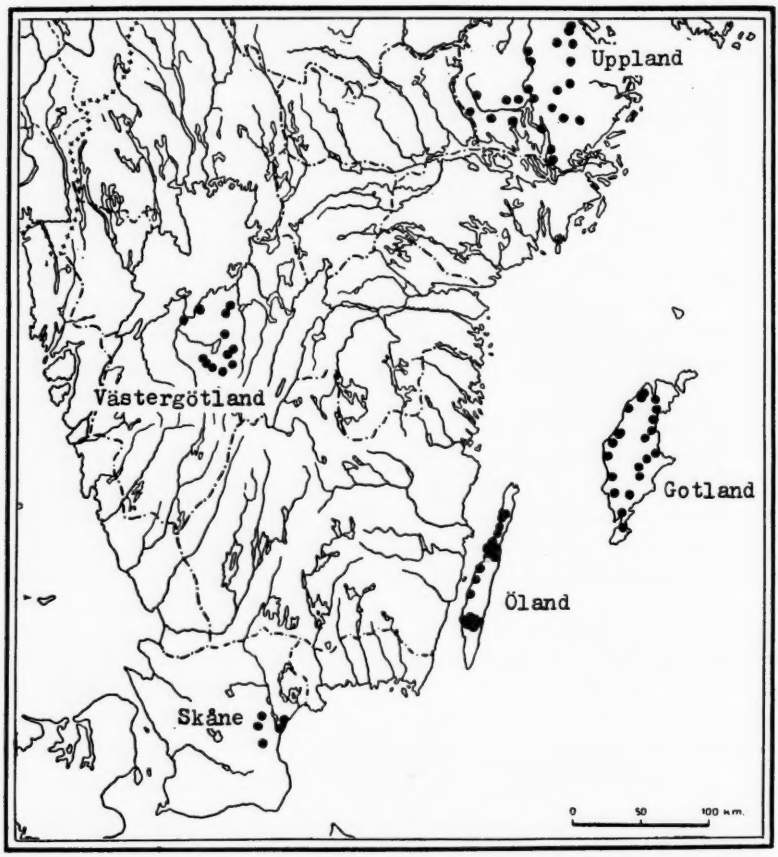


Map. 1. The distribution of *Phleum phleoides* (L.) KARST. (*Phleum Boehmeri* WIB.) in Scandinavia. From E. HULTÉN: Atlas över växternas utbredning i Norden (Atlas of the distribution of vascular plants in N. W. Europe).

be triploid and two tetraploid. The occurrence of triploids and tetraploids is explained by the presence of tetraploid pollen mother cells in some plants (see page 453), diploid gametes probably being produced in low frequency also on the female side. From the data it is concluded that in Sweden *Phleum phleoides* is present only as a diploid.

The frequency of accessory chromosomes is given in Table 2. Here the triploid and tetraploid plants have been left out. The distribution of the localities can be seen in Map 2.

The over-all frequency of plants with accessory chromosomes is 31,1 per cent with an average number of accessory chromosomes per plant amounting to 0,63. There is a marked heterogeneity within as well as between areas. Thus the difference between Öland and all other areas with respect to frequency of accessory chromosomes is statistically



Map 2. Location of the sampled populations of *Phleum phleoides* in Sweden.

significant. Also between these other areas the statistical analysis indicates heterogeneity.

As the distribution of *Phleum phleoides* in Sweden is discontinuous and probably little or no communication exists between populations in different areas, it is possible that the differences with respect to the frequency of accessory chromosomes are mainly due to phyto-historical factors or chance distribution. There is also the possibility that the outcome of natural selection in this respect is influenced by edaphic, climatic and biotic differences between areas. The present study does

TABLE 2. *Frequency of plants with different numbers of accessory chromosomes in natural populations of Phleum phleoides in Sweden.*

Area	Sample Nos.	Number of plants with respective numbers of accessory chromosomes									Total number of plants	% plants with acc. chr.	Average number of acc. chr. per plant
		0	1	2	3	4	5	6	7	8			
Skåne	9	19	3	2[1]	—	—	—	—	—	—	24	20,8	0,29
	13	10	—	1	—	—	—	—	—	—	11	9,1	0,18
	16	—	4[1]	5	7	3	1	—	—	—	20	100,0	2,60
	105	—	1	5	1	1	—	—	—	—	8	100,0	2,25
	106	11	3	—	2	—	—	—	—	—	16[2]	31,3	0,56
Total: Skåne		40	11	13	10	4	1	—	—	—	79	49,4	1,11
Väster-götland	29	13	2	6	—	—	—	—	—	—	21	38,1	0,67
	32	3	—	1	—	—	—	—	—	—	4	(25,0)	(0,50)
	36	18	4	1	1	—	—	—	—	—	24	25,0	0,38
	38	8	4	3	3	2	—	—	—	—	20	60,0	1,35
	39	3	—	—	—	—	—	—	—	—	3	(0,0)	(0,0)
	46	13	2	2	—	—	—	—	—	—	17	23,5	0,35
	47	12	1	—	—	—	—	—	—	—	13	7,7	0,08
	49	8	6	1	1	—	—	—	—	—	16	50,0	0,69
	52	10	—	2	—	—	—	—	—	—	12	16,7	0,23
	53	15	3	3	—	—	—	—	—	—	21	28,6	0,43
	54	13	1	2	—	—	—	—	—	—	16	18,8	0,31
	55	13	5	3	1	1	—	—	—	—	23	43,5	0,78
Total: Väster-götland		129	28	24	6	3	—	—	—	—	190	32,1	0,56
Öland	62	20	1	—	—	—	—	—	—	—	21	4,8	0,05
	64	13	—	1	—	—	—	—	—	—	14	7,1	0,14
	65	19	—	1	—	—	—	—	—	—	20	5,0	0,10
	66	13	2	1	1	—	—	—	—	—	17	23,5	0,41
	67	9	3	2	—	—	—	—	—	—	14	35,7	0,50
	68	17	3	—	—	—	—	—	—	—	20	15,0	0,15
	69	20	—	1	—	—	—	—	—	—	21	4,8	0,10
	70	20	2	3	—	—	—	—	—	—	25[3]	20,0	0,32
	71	17	1	—	—	—	—	—	—	—	18	5,6	0,06
	72	19	—	—	—	—	—	—	—	—	19	0,0	0,0
	73	12	4	2	—	—	—	—	—	—	18	33,3	0,44
	74	13	2	4	—	—	1	—	—	—	20	35,0	0,75
	75	16	—	—	—	—	—	—	—	—	16	0,0	0,0
	76	18	—	2	—	—	—	—	—	—	20	10,0	0,20
	77	18	—	—	—	—	—	—	—	—	18	0,0	0,0
	78	21	—	—	—	—	—	—	—	—	21	0,0	0,0
	79	8	—	—	—	—	—	—	—	—	8	0,0	0,0
	80	15	—	—	—	—	—	—	—	—	15	0,0	0,0
Total: Öland		288	18	17	1	—	1	—	—	—	325	11,4	0,18

Table 2 (continued)

Area	Sample Nos.	Number of plants with respective numbers of accessory chromosomes									Total number of plants	% plants with acc. chr.	Average number of acc. chr. per plant
		0	1	2	3	4	5	6	7	8			
Uppland	81	7	2	2	1	1	—	—	—	—	13	46,2	1,00
	82	13	4	—	—	—	—	—	—	—	17	23,5	0,24
	83	12	—	—	—	—	—	—	—	—	12	0,0	0,0
	84	8	1	4	—	—	—	—	—	—	13	38,5	0,69
	85	5	5	4	2	1	—	1	1	—	19	73,7	1,89
	86	12	—	4[1]	2	1	—	—	—	—	19	36,7	0,95
	87	11	3	6	—	—	—	—	—	—	20	45,0	0,75
	88	2	2	2	—	—	—	—	—	—	6	66,7	1,00
	89	7	4	5	3	1	—	—	—	—	20	65,0	1,35
	90	6	2	4	4	—	1	—	—	—	17	64,7	1,59
	91	16	—	3	1[4]	—	—	—	—	—	20	20,0	0,45
	92	15	1	4	2	—	—	—	—	—	22[2]	31,8	0,68
	93	13	1	2	1	—	—	—	—	—	17	23,5	0,47
	94	17	1	1	1	—	—	—	—	—	20	15,0	0,30
	95	12	4	1	1	—	—	—	—	—	18	33,3	0,50
	96	2	7	2	2	—	1[5]	—	1	—	15	86,7	1,93
	97	11	6	2	3	—	—	—	—	—	22	50,0	0,86
	98	17	2	1	1	—	—	—	—	—	21	19,0	0,33
	99	15	2	1	1	—	—	1	—	—	20	25,0	0,65
	100	2	—	1	1	3[6]	5	1	1	1[7]	15	86,7	4,20
	101	11	7	2	1	—	—	—	—	—	21	47,6	0,67
	102	8	3	4	2	1	—	1	—	—	19	57,9	1,42
	103	16	2	1	—	—	—	—	—	—	19	15,8	0,21
	104	5	6	3	4	—	—	—	—	—	18	72,2	1,33
Total: Uppland		243	65	59	33	8	7	4	3	1	423	42,6	0,95
Gotland	107	13	3	3	1	—	—	—	—	—	20	35,0	0,60
	108	13	5	1	—	—	—	—	—	—	19	31,6	0,37
	109	15	4	1	2[8]	—	—	—	—	—	22[2]	31,8	0,55
	110	9	3	6[1]	1	—	—	—	—	—	19	52,6	0,95
	111	10	1	5	2	2	—	—	—	—	20	50,0	1,25
	112	13	4	2	—	—	—	—	—	—	19	31,6	0,42
	113	12	4	1	—	1	—	—	—	—	18	33,3	0,56
	114	21	1	—	—	—	—	—	—	—	22	4,5	0,05
	115	12	4	2[9]	—	—	—	—	—	—	18	33,3	0,44
	116	12	1	—	—	—	—	—	—	—	13	7,7	0,08
	117	18	—	1	—	—	—	—	—	—	19	5,3	0,11
	118	11	2	1	2	2	1	—	—	—	19	42,1	1,21
	119	17	1	2	—	—	—	—	—	—	20	15,0	0,25
	120	2	4	7	3	1	—	—	—	—	17	88,2	1,82
	121	9	4	4	4	1	—	—	—	—	22	59,1	1,27
	122	19	1	1	—	—	—	—	—	—	21	9,5	0,14
	123	12	2	1	—	—	—	—	—	—	15	20,0	0,27

Table 2 (continued)

Area	Sample Nos.	Number of plants with respective numbers of accessory chromosomes									Total number of plants	% plants with acc. chr.	Average number of acc. chr. per plant
		0	1	2	3	4	5	6	7	8			
	124	22	1	—	—	—	—	—	—	—	23	4,3	0,04
	125	11	3	4	—	1	—	—	—	—	19	42,1	0,79
	126	15	3	2	—	—	—	—	—	—	20	25,0	0,35
Total: Gotland		266	51	44	15	8	1	—	—	—	385	30,9	0,57
Total all samples		966	173	157	64	24	10	4	3	1	1402	31,1	0,63

[1] In one of the plants the acc. were of the large type
[2] In addition one tetraploid plant with acc. chr.
[3] In addition one triploid plant with acc. chr.
[4] The plant had two st. acc. and one s. acc.
[5] » » » four » » » » » »
[6] One » » two » » » two » »
[7] » » » six » » » » » »
[8] » » » two » » » one l. »
[9] » » » one » » » » s. »

Analysis of variance of the data from Table 2 based on the mean values for the individual populations.

Cause of var.	Degrees of freedom	Sum of squares	Mean square	Quotient	p-value
Between areas	4	8,3674	2,0919	5,2772	0,001
» Öland and remaining areas	1	4,7951	4,7951	12,0966	0,001
» »remaining areas» ..	3	3,5723	1,1908	3,0040	0,05—0,01
Within areas	74	29,3301	0,3964	—	—
Total	78	37,6975	—	—	—

not permit any definite conclusions to be drawn with respect to this question, but indications of differences in frequency of accessory chromosomes in various parts of the island of Öland direct attention to the significance of ecologic factors.

The island of Öland, in spite of its small size, has rather variable edaphic conditions. Thus, in the southern part of Öland there is a large area of poor pasture land, mainly on calcerous bedrock. The layer of soil is very thin and the rock is often exposed. The area referred to here is the largest of several such areas on the island of Öland. These limestone pavements, which have a very characteristic flora, are in Swedish called »alvar». The alvar area in Southern Öland being the largest is

called the Large Alvar. In the northern part of the island there are several smaller alvar areas scattered along the western coast. In contrast to the large Alvar, the northern alvar areas were covered by the sea during the Ancylus and Litorina Epochs in post-glacial times, and the thin layer of sandy soil is less rich in mould and lime than is generally the case in the Large Alvar. This is also reflected in the flora which is represented by a lesser number of species in the northern areas (STERNER, 1938). All alvar areas are badly drained owing to the slight inclination, large parts of them being regularly flooded.

Besides the alvar regions with their special characteristics, areas with marine deposits of sand and gravel, moraine soils of different types and peat soils are scattered over the island. Unfortunately, the sampled populations are not representative for the entire area, most localities being situated on alvar ground, only a few on sandy, gravelly soils outside the alvar.

TABLE 3. *Frequency of plants with accessory chromosomes in different areas on the island of Öland.*

Areas	Number of plants with respective numbers of accessory chromosomes						Total number of plants	% plants with acc. chr.	M. acc. chr. per plant
	0	1	2	3	4	5			
Alvar areas on northern Öland. Loc. 62—65, 68—72	145	7	6	—	—	—	158	8,2	0,12
Sandy, gravelly loc. not of alvar type. Loc. 66, 67, 73, 74	47	11	9	1	—	1	69	31,9	0,54
The Large Alvar. Loc. 75—80	96	—	2	—	—	—	98	2,0	0,04
Total	288	18	17	1	—	1	325	11,4	0,18

From Tables 2 and 3 it can be seen that the samples from the Large Alvar are almost entirely lacking in accessory chromosomes, whereas in the northern alvar areas there is on an average a low frequency of plants with accessories. Finally, in the few populations growing outside the alvar, accessory chromosomes are very common. Although insufficient, the data suggest that plants with accessory chromosomes are most common in populations growing on light, well-drained soils. In all the main areas except Öland and a number of small alvar-like areas in Västergötland and Gotland, the species is nearly exclusively confined to such soils. The situation might thus be fundamentally the same as that in *Festuca pratensis*, where in Sweden accessory chromosomes are most common in populations growing on rich, clayey soils, which are also the

most favourable for the species (BOSEMARK, 1956 a). Further studies are, however, necessary to decide whether ecologic conditions have influenced the frequency and distribution of accessory chromosomes in *Phleum phleoides* or not. As the species has a more or less continuous distribution on the island of Öland, this area would be suitable for such a study.

IV. CYTOLOGICAL INVESTIGATIONS

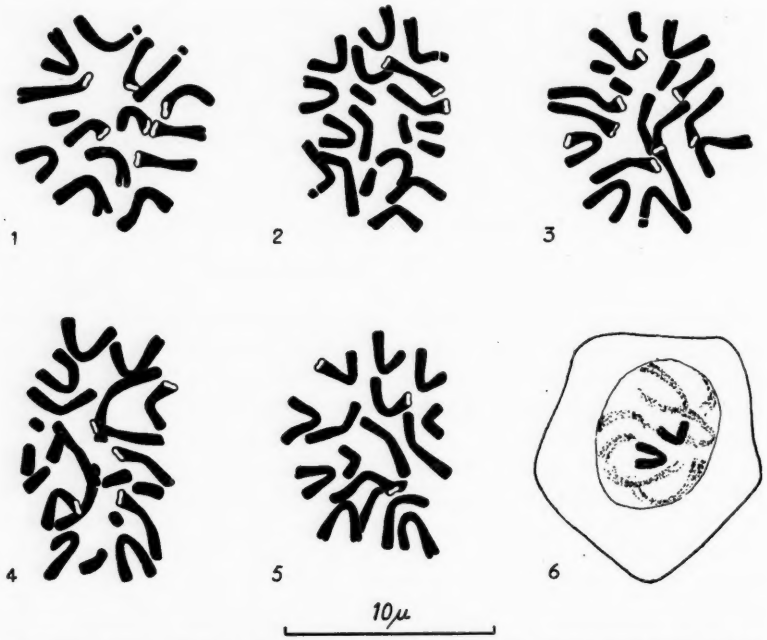
1. The somatic chromosomes

The appearance of the normal chromosome complement as well as that of the accessory chromosomes is seen in Figs. 1—5. As in the material studied by BÖCHER (1950), different types of accessory chromosomes have been found in the material collected in Sweden. They have been classified as follows:

(a) *The standard type* (st. acc.) Figs. 1, 2. In 98 per cent of all plants with accessory chromosomes this type only was found. The length of the standard type is about $^2/_3$ of the average for the normal chromosomes. The centromere has a sub-terminal position but is generally not clearly seen in the root-tip metaphase plates. At pachytene of meiosis and at metaphase of the first pollen mitosis, however, the position of the centromere can easily be determined (Figs. 7—9, 33).

(b) *Small accessory chromosomes* (s. acc.) Figs. 3, 4. In five plants accessory chromosomes smaller than the standard type have been found. They all seem to have a more or less median centromere. Two of these plants were studied at pachytene. In one of them with $2n=14+$ $+$ (2 st.$+$2 s.) the small accessory chromosomes had lost part of the long arm of the standard type, thus constituting deficiency chromosomes. In the other plant with $2n=14+$ (6 st.$+$2 s.) the small accessories were found to be iso-chromosomes representing twice the short arm of the standard type (Figs. 18, 19).

(c) *Large accessory chromosomes* (l. acc.) Fig. 5. Accessory chromosomes larger than the standard type have been found in five plants. They all have a median centromere, the arms being of the same length as the long arm of the standard type. One plant with $2n=14+$ $+$ (2 st.$+$1 l.) could be studied at pachytene and here the pairing behaviour of the large accessory chromosome showed that it is an iso-chromosome consisting of twice the long arm of the standard type (Figs. 10, 20, 21). It is likely that also the other large accessory chromosomes are iso-chromosomes of the same type.



Figs. 1—5. Somatic metaphase plates in root-tips from plants with a varying number of accessory chromosomes of different types. — Fig. 1, $2n=14+1$ st. acc.; Fig. 2, $2n=14+$ $+5$ st. acc.; Fig. 3, $2n=14+2$ st. acc. $+2$ s. acc. (The s. acc. is the one described in the text which has originated through a terminal deficiency in the long arm of the st. acc.); Fig. 4, $2n=14+6$ st. acc. $+2$ s. acc. (The s. acc. are iso-chromosomes consisting of the small arm of the st. acc.); Fig. 5, $2n=14+2$ l. acc.; Fig. 6, prophase in a plant with $2n=14+2$ l. acc. showing positive heteropycnosis of large accessory chromosomes.

In resting nuclei and at early prophase in root-tips the amount of positively heteropycnotic material increases with a corresponding increase in the number of accessory chromosomes. This clearly suggests that the accessory chromosomes in *Phleum phleoides* are heterochromatic. In plants with one or two accessory chromosomes of the standard type, it is, however, difficult to identify these in resting nuclei or at very early prophase. This depends on the fact that at least some of the normal chromosomes, besides intercalary heterochromatic segments on each side of the centromere, have big knobs at the chromosome ends. These obviously often fuse to form chromocentra of about the same size as a standard accessory chromosome. In plants with large accessory

chromosomes, however, these are generally clearly seen at early prophase (Fig. 6). Thus there is no doubt that the accessory chromosomes in *Phleum phleoides* are heterochromatic in the morphological sense of the term.

The number of accessory chromosomes in the root-tips has always been found to be constant within an individual, and with the exception of a diploid-tetraploid chimera no verified cases of somatic variation have been found either within or between the roots from one and the same plant. This also holds true for the small and large types.

2. Meiosis

Meiosis has been studied in greater detail in 21 plants with the following numbers and types of accessory chromosomes.

TABLE 4. *Number of plants with respective numbers and types of accessory chromosomes studied in meiosis.*

Number and types of acc. chr.	No acc. chr.	1 st.	2 st.	3 st.	4 st.	5 st.	7 st.	2 s. + 2 st.	2 s. + 6 st.	1 l.	1 l. + 2 st.	2 l.	Total
Number of plants	3	2	5	1	1	1	1	1	1	1	1	3	21

In the majority of the plants the behaviour of the normal chromosomes was on the whole regular, but univalents, non-orientated bivalents and irregular anaphase separation were observed in a low but varying frequency in some plants. Three plants were heterozygous for a translocation, rings and chains of four chromosomes being present in low frequency; and in five plants anaphase bridges and fragments indicated heterozygosity for one or more inversions. Syncyte formation in low frequency was observed in three plants. Most of the syncytes contained the tetraploid number, and at metaphase I all the chromosomes were situated on the same spindle. At diakinesis two different groups were sometimes seen and might result in syncytes with more than one spindle as described by BÖCHER (1950). In a few cases the two groups of chromosomes in a bi-nucleate pollen mother cell were in different stages of meiosis, for instance, one in diakinesis, the other in pachytene or an earlier stage.

The chiasma frequency as determined at metaphase I varied highly between plants, some having nearly only rod bivalents, whereas in others most bivalents had two chiasmata. The number of accessory

chromosomes in the pollen mother cells has always corresponded to that found in the root-tips from one and the same plant, and with the exception of the isolated cases of syncyte formation not a single case of variation in number of accessory chromosomes between pollen mother cells within a plant was observed.

The accessory chromosomes in *Phleum phleoides* are clearly heterochromatic also at the meiotic prophase. At pachytene it can be seen that both arms of the standard type are composed of highly heterochromatic segments. At diplotene and diakinesis the heterochromaty is manifested in more even outlines, a more compact appearance than the normal chromosomes and a tendency to stickiness. The accessory chromosomes have never been seen to pair with members of the normal complement. The appearance of bivalents of standard accessory chromosomes at different stages of early prophase is seen in Figs. 7—9, 17. In plants with higher numbers of standard accessory chromosomes, multivalents, mainly tetravalents, have been observed at pachytene. Also at diplotene and diakinesis high associations are common in these plants, but generally most of them break up to bivalents and univalents before metaphase I is reached.

At metaphase I univalent standard accessory chromosomes are generally situated at one of the poles. When located close to the equatorial plane and left between the poles at anaphase I, the univalent generally divides. The daughter univalents seem practically always to reach the telophase nuclei and are thus very seldom seen at late telophase I—interphase. Accessories not dividing at anaphase I divide regularly at anaphase II, whereas those which have divided at the first division are probably distributed at random at the second division.

In four of the five plants with two standard accessory chromosomes studied, these formed a bivalent with surprising regularity. Thus, in only about 2 per cent of the cells at diakinesis were the accessory chromosomes unpaired. Also at metaphase I the pairing was very regular (Table 5). Consequently, anaphase I was regular in these plants; uneven distribution and lagging univalents were seen in only a few cases. The subsequent stages of meiosis were normal, and tetrad micronuclei practically absent.

Also in the fifth plant the standard accessory chromosomes regularly formed a bivalent. However, these sometimes had difficulties in dividing at anaphase I resulting in the formation of bridges and drawn-out chromatid ends. In no case could an accompanying fragment be observed; and studies of good pachytene slides (Fig. 8) showed homo-

Figs. 7—9. Paired standard accessory chromosomes at pachytene. — Fig. 10, configuration at pachytene in a plant with $2n=14+2$ st. acc.$+1$ l. acc. The l. acc. is an iso-chromosome consisting of twice the long arm of the st. acc. It is suggested that the configuration is the result of inter-arm pairing in the iso-chromosome in combination with pairing between homologous segments in the three chromosomes (see Fig. 21). — Fig. 11, paired st. acc. at diplotene. Note the more compact appearance of the acc. chr. compared to that of the normal chromosomes. — $\times 3800$.

TABLE 5. *Pairing of standard accessory chromosomes at metaphase I in plants with 2n=14+2 st. acc.*

Plant Nos.	Number of cells with respective configurations		Total number of cells
	1_{II}	2_I	
646	116	13	129
1039	44	—	44
1321	68	1	69
Total	228	14	242

logous pairing of the accessories without a chromatid loop. Thus, the bridges are probably not the result of a chiasma within an inverted segment in one of the accessory chromosomes but more likely due to pseudo-chiasmata between non-sister-chromatids. In other respects this plant did not deviate from the others.

At metaphase I, in a plant with three standard accessory chromosomes, 47 cells were analysed. 38 of these showed $1_{II}+1_I$, 8 cells had 3_I and, finally, in 1 cell, the accessory chromosomes formed a trivalent. At anaphase II laggards were common but at early telophase II most of these had reached the nuclei. Only 5 per cent of the tetrads had a micronucleus in one of the tetrad cells.

In the plant with $2n=14+4$ st. all four accessory chromosomes were associated in 65 per cent of the cells studied at diakinesis. At metaphase I, associations of four were still present in about 30 per cent of the cells, 60 per cent had two bivalents and in the rest there were two or more univalents. At later stages the behaviour of the accessory chromosomes was the same as previously described.

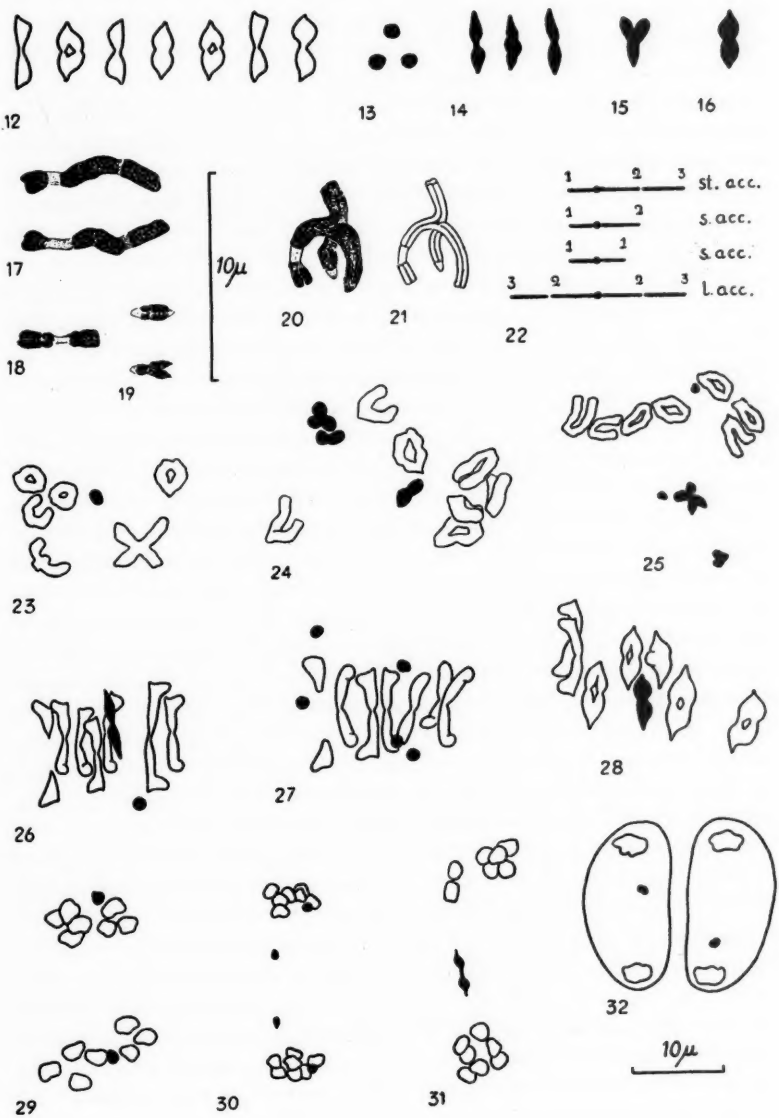
Also in the plants with five and seven standard accessory chromosomes high associations were observed at diakinesis. In the plant with seven accessory chromosomes, for instance, 13 cells out of 35 showed associations of five or more accessory chromosomes. At metaphase I, however, this plant had a very high frequency of univalents, and higher configurations than bivalents were seen only in a few cases and even these were not quite indisputable. In spite of this, micronuclei were seldom found at telophase I or at the tetrad stage.

Of the two plants with small accessory chromosomes studied at meiosis, one had two standard and two small accessory chromosomes, the other having six standard and two small. In the first-mentioned plant studies of pachytene revealed that the small accessory was a deficiency chromosome having lost about half the long arm of the

standard type (Fig. 22). The pairing of the small accessories at diakinesis and metaphase was less regular than for the standard ones. In 57 out of 65 cells at metaphase I the standard accessories formed a bivalent whereas the corresponding figure for the small was 40. In other respects the behaviour of the two types did not differ. At pachytene, in the plant with $2n=14+(6\text{ st.}+2\text{ s.})$, evidence was obtained that the small accessory chromosomes were iso-chromosomes consisting of twice the short arm of the standard type. In most cells at early prophase they were present as separate bodies showing inter-arm pairing (Fig. 19), but a few cases of inter-chromosome pairing were also seen (Fig. 18). At diakinesis the small accessory chromosomes were unpaired in 35 out of 45 cells analysed. Although the standard accessories present often formed high associations at diakinesis, the small accessory chromosomes were not associated with these in more than 2 of the 45 cells. At metaphase I the small accessory chromosomes were practically always unpaired and situated outside the metaphase plate. From later stages of meiosis only few observations could be made, and the behaviour of the small accessory chromosomes could thus not be followed in detail.

Of the four plants with large accessory chromosomes studied, only the plant with $2n=14+(2\text{ st.}+1\text{ l.})$ gave fairly satisfactory pachytene slides. In a number of cells it could be seen that the two arms of the large accessory chromosome had paired with each other. More frequent, however, were associations between the two standard accessory chromosomes and the large one. In some cases the configurations indicated true pairing between homologous segments in the three chromosomes

Figs. 12—32. Meiosis in plants with different types of accessory chromosomes. — Fig. 12, bivalents from the normal complement at metaphase I, separately drawn; Figs. 13—15, different configurations of st. acc. at metaphase I, univalents, bivalents and a trivalent; Fig. 16, bivalent formed by two l. acc.; Fig. 17, paired st. acc. at late pachytene; Figs. 18—19, the small iso-chromosome at pachytene; Fig. 18, two s. acc. showing inter-chromosome pairing; Fig. 19, s. acc. showing inter-arm pairing; Fig. 20, configuration formed by two st. acc. and one l. acc. of iso-chromosome type; Fig. 21, schematic drawing showing the mode of pairing between the three chromosomes in Fig. 20; Fig. 22, schematic representation of the different types of accessory chromosomes found; Fig. 23, diakinesis in a plant with $2n=14+1$ st. acc. The plant is heterozygous for a translocation; Fig. 24, diakinesis in a plant with $2n=14+5$ st. acc.; Fig. 25, diakinesis in a plant with $2n=14+6$ st. acc. $+2$ s. acc. The s. acc. are those seen in Figs. 18—19; Figs. 26—28, metaphase I in plants with $2n=14+3$ st. acc., $2n=14+5$ st. acc. and $2n=14+2$ l. acc., respectively; Fig. 29, anaphase I in a plant with $2n=14+2$ st. acc.; Fig. 30, dividing univalent at anaphase I in a plant with $2n=14+3$ st. acc.; Fig. 31, dividing l. acc. chr. at anaphase I in a plant with $2n=14+$ $+1$ l. acc.; Fig. 32, lagging st. acc. at late anaphase I in a plant with $2n=14+3$ st. acc. — Figs. 17—19, 25, 30 and 31 were drawn from Feulgen stained squashes, the others from fresh aceto-carmine squashes.


12
13
14
15
16
17
18
19
10μ
20
21
1 2 3 st. acc.
1 2 s. acc.
1 1 s. acc.
3 2 2 3 L. acc.
22
23
24
25
26
27
28
29
30
31
32
10μ

(Fig. 20), but generally it could not be decided whether the associations were the result of such pairing or of an unspecific clumping together.

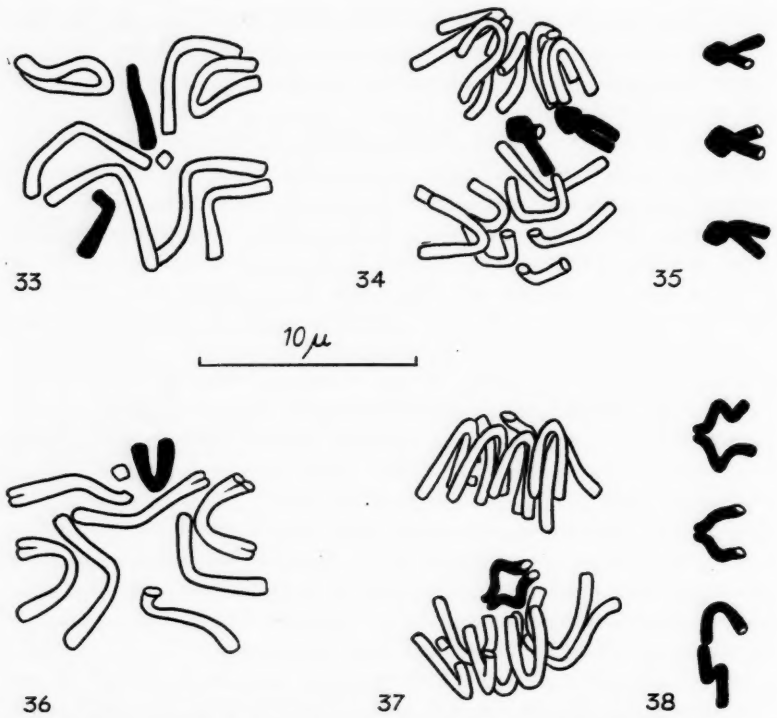
Although the morphology of the large accessory chromosome could not be studied in detail, there is hardly any doubt that it is an isochromosome representing a duplication of the long arm of the standard accessory chromosome and that it has arisen by mis-division in the centromere of the latter. As all large accessory chromosomes have been found to be of the same size and they all have a median centromere, it is likely that they have all originated in the same way. In two plants with $2n=14+2$ l. the pairing of the large accessory chromosomes was studied at diakinesis and metaphase I. In one of the plants they were nearly always associated at diakinesis, and even at metaphase I they were observed to form a bivalent in about 90 per cent of the cells. In the other plant the accessory chromosomes were univalent in 30 per cent of the cells studied at diakinesis, the corresponding figure for metaphase I being about 50 per cent. In correspondence with this, the first-mentioned plant had few lagging univalents at anaphase I, whereas in the other plant 30 per cent of the first anaphases showed one or two laggards. Even at late telophase I lagging univalents were still seen in the process of dividing in about 10 per cent of the cells. At the corresponding stage in plants with standard accessory chromosomes generally all univalents have divided and the daughter univalents are included in the telophase nuclei. This indicates that the univalent large accessory chromosome might have greater difficulties in dividing at anaphase I than has the standard type. This conclusion is strengthened by the fact that in the plant with $2n=14+1$ l. (plant 49), this tendency was still more pronounced. Thus, at anaphase I the accessory chromosome was left between the poles in 64 per cent of the cells. At late telophase I approximately 30 per cent of the cells still showed lagging univalents. Whether this property of the large accessory chromosomes results in a higher degree of meiotic elimination than is generally the case with the standard type could not be decided from the cytological studies, but the results of the crosses point to this possibility. At later stages of meiosis the behaviour of the large accessory chromosome could not be seen to deviate from that of the standard type. No clear cases of neo-centric activity of chromosome ends as described for the accessory chromosomes in *Festuca pratensis* (BOSEMARK, 1956 b) have been observed in *Phleum phleoides*. As the centromere of the standard type has a sub-terminal position, and, in addition, the chromosomes are small, such a phenomenon will also be difficult to detect here. In the large type the attenuated ends of

dividing univalents strikingly resemble the pictures seen in *Festuca pratensis*, but as no indisputable case of separated chromosome ends directed towards the poles has been obtained the question has to be left open.

3. Pollen mitosis

The first pollen mitosis has been studied in plants with standard as well as large accessory chromosomes. Both types have been found to be capable of undergoing directed non-disjunction at anaphase of this division. The underlying mechanism is the same as in the earlier reported cases of directed non-disjunction in grasses (for a review see BOSEMARK, 1954 a and 1956 a). Thus, in the standard type the two chromatids are held together at two symmetrically situated points on each side of the centromere (Figs. 34, 35). As in rye (MÜNTZING, 1946) the sub-terminal position of the centromere gives the chromosome the appearance of a bivalent with two localized chiasmata and two free arms. In contrast to the situation in *Festuca*, where the accessory chromosome is generally easily seen between the two separating anaphase groups, only later on being included in the generative nucleus, in *Phleum* the standard accessory chromosome is often difficult to observe. Already at the beginning of the anaphase movement it is generally hidden by the normal chromosomes. Sometimes the spindle is also bent and the anaphase groups placed at right angles to each other, which renders observation still more difficult. This probably depends on the position of the spindle at the first pollen mitosis being more asymmetrical in *Phleum phleoides* than in *Festuca pratensis*, which in its turn leads to a more pronounced asymmetry of the spindle itself. Thus the group of chromosomes giving rise to the generative nucleus moves very little during anaphase, and the accessory chromosome which is situated in the equatorial plane is practically immediately included in the generative nucleus. From the point of view of the accessory chromosome this should be an advantage, and as a matter of fact not a single case of attenuated chromatids and failing non-disjunction was observed for the standard type. However, only a few plants were studied, and none with more than four accessory chromosomes; the observations should thus not be generalized.

The non-disjunction mechanism of the large type was studied in a plant with two such chromosomes. On the basis of the suggested origin of the large accessory chromosomes one might have expected that the separation of the chromatids would be prevented in two regions close to the centromere, leaving four free chromatid ends. However, in no case



Figs. 33—38. The first pollen mitosis in plants with accessory chromosomes. — Fig. 33, pollen metaphase plate from a plant with $2n=14+4$ st. acc.; Fig. 34, anaphase from the same plant, showing non-disjunction of the accessories; Fig. 35, configurations of st. acc. at anaphase, separately drawn; Fig. 36, pollen metaphase plate from a plant with $2n=14+2$ l. acc.; Fig. 37, anaphase from the same plant; Fig. 38, configurations of l. acc. at anaphase, separately drawn.

was this figure observed. Instead, the chromatids were generally very loosely attached rather close to the ends (Figs. 37, 38). In some cases it could nevertheless be seen that the primary points of attachment are very probably intercalary segments, but that generally these points cannot resist the pull of the centromeres and consequently break up, giving the impression of slipping along the arms. Thus, in the plant studied the non-disjunction of the large accessory chromosomes seems to be less effective than in the standard type. With the knowledge of the variation in the effectiveness of the non-disjunction mechanism in *Festuca pratensis* (BOSEMARK, 1954 a and b), it is, however, probable that in other

plants with large accessory chromosomes the behaviour is more in line with that of the standard type. This is also indicated by the results of the crosses.

V. INHERITANCE OF THE ACCESSORY CHROMOSOMES

The standard type. — In Table 6 the results of crosses involving only standard accessory chromosomes are given. As is immediately seen there is no tendency to a numerical increase of the accessory chromosomes on the female side. On the male side, on the other hand, the data confirm the cytological observations about the directed non-disjunction of the accessories at the first pollen mitosis. In the two reciprocal crosses the regularity of meiosis as well as of the non-disjunction is reflected. In plant 647 this was also cytologically verified. In the present plants the non-disjunction mechanism in combination with the slight meiotic elimination will evidently result in a very effective spreading of the accessory chromosomes.

TABLE 6. *Inheritance of the standard type of accessory chromosome.*

Crosses	Parents Plant Nos. ♀	♂	Number of acc. chr. ♀	♂	F_1 Number of acc. chr. 0	1	2	Number of plants	M. acc. chr. per plant
K 2/55	327	288	1	0	26	19	—	45	0,42
K 14/55	706	6	2	0	16	61	3	80	0,84
K 13/55	6	706	0	2	3	1	39	43	1,84
K 22/55	647	1388	2	0	1	34	—	35	0,97
K 21/55	1388	647	0	2	1	—	40	41	1,95

Inheritance of a small accessory chromosome. — In Table 7 the result of a reciprocal cross involving a plant with two standard and two small accessory chromosomes is given. The small accessory chromosome in this plant is the one, previously mentioned, which has lost part of the long arm of the standard type. The separate analyses show no marked differences between the two types with respect to either meiotic elimination or effectiveness of the non-disjunction mechanism. It is possible that the meiotic elimination is slightly higher for the small type, but the number of progeny plants is not sufficient to decide this. From the data it is evident that the small accessory chromosome, even when present alone in the pollen grain, is capable of undergoing non-disjunction. It cannot be decided whether the presence of standard accessory chromosomes together with small ones in the same pollen grain affects the latter

TABLE 7. *Comparison between the inheritance of the standard accessory chromosome and a small accessory chromosome which has originated through a terminal deficiency in the long arm of the standard.*

A. Observed chromosome numbers

Crosses	Parents Plant Nos. ♀	♂	Number of acc. chr. ♀	♂	F_1 Number of acc. chr. 1 st.	2 st.	1 s.	2 s.	1 st. + 1 s.	1 st. + 2 s.	2 st. + 1 s.	2 st. + 2 s.	2 st. + 4 s.	4 st. + 2 s.	Number of plants
K 7/55	942×	57	(2 st.+2 s.)×0		5	2	1	1	52	3	1	—	—	—	65
K 8/55	57×	942	0×(2 st.+2 s.)		—	8	2	4	1	1	—	30	1	1	48

B. Analysis of the inheritance of the standard accessory chromosome

Parents Number of acc. chr.	F_1 Number of st. acc. chr. 0	1	2	3	4	Number of plants	M. st. acc. chr. per plant
(2 st.+2 s.)×0	2	60	3	—	—	65	1,02
0×(2 st.+2 s.)	6	2	39	—	1	48	1,75

C. Analysis of the inheritance of the small accessory chromosome

Parents Number of acc. chr.	F_1 Number of s. acc. chr. 0	1	2	3	4	Number of plants	M. s. acc. chr. per plant
(2 st.+2 s.)×0	7	54	4	—	—	65	0,95
0×(2 st.+2 s.)	8	3	36	—	1	48	1,65

TABLE 8. *Inheritance of the large type of accessory chromosome.*

Crosses	Parents Plant Nos. ♀	♂	Number of acc. chr. ♀	♂	F_1 Number of acc. chr. 0	1	2	Number of plants	M. l. acc. chr. per plant
K 19/55	49×	1387	1 l.×0		18	6	—	24	0,25
K 20/55	1387×	49	0×1 l.		53	—	10	63	0,32
K 18/55	1148×	1140	0×2 l.		4	1	14	19	1,53

in such a way as to make them more liable to undergo non-disjunction, a phenomenon observed in *Festuca pratensis*. The results indicate that no segment necessary for the non-disjunction is situated in the distal part of the long arm of the standard type.

Inheritance of the large type. — The results of the crosses involving the large type of accessory chromosomes (Table 8) are rather uncertain due to the small number of progeny plants. The cross involving a plant with only one such chromosome indicates a marked meiotic elimination.

The cytological examination of this plant showed a high frequency of lagging univalents at very late telophase I. If the two chromatids are unable to separate in time, the accessory chromosome might thus be eliminated already at the first division, although probably in most cases the elimination takes place during the second division. The mechanism of non-disjunction of the large accessory chromosomes in the plants studied here does not seem to be inferior to that of the standard type. As pointed out earlier, it is very likely that all the large accessory chromosomes are iso-chromosomes representing twice the long arm of the standard type. If this supposition is correct, the loss of the short arm of the standard accessory chromosome does not seem to affect the non-disjunction mechanism. The findings that neither the loss of the distal part of the long arm nor the short arm influence the capacity for non-disjunction, suggest that the segment responsible for this phenomenon is situated in the proximal part of the long arm. The role of the centromere in the manifestation of the non-disjunction cannot be assessed, but it is quite likely that the process is the result of an interaction between the centromere and a specialized segment.

VI. DISCUSSION

The analysis of the material collected has revealed that the frequency of plants with accessory chromosomes in natural populations of *Phleum phleoides* in Sweden is definitely higher than in *Festuca pratensis* (BOSEMARK, 1956 a), approaching the values found for *Centaurea scabiosa* (FRÖST, personal communication and FRÖST in MÜNTZING, 1954). In the author's opinion this makes it necessary to look upon the accessory chromosomes in *Phleum phleoides* as normal, although dispensable, constituents of the genome. Recently DARLINGTON (1956) has suggested that accessory chromosomes, or B-chromosomes, might increase the variability and adaptability of a species, and that their value to the species might be more experimental than intrinsic. Although these ideas are speculative and lack experimental evidence, they are useful in so far as they broaden the view on problems concerning accessory chromosomes. With the increased knowledge of the occurrence of accessory chromosomes in the plant as well as the animal kingdom, it is no longer justifiable to look upon such cases as isolated cytological oddities without evolutionary consequences and significance. Also the theory of the parasitic nature of accessory chromosomes (ÖSTERGREN, 1945, 1947) has lost much of its likelihood in the light of recent investigations on ac-

cessory chromosomes in widely different species. These questions will be discussed in greater detail in a following paper.

As pointed out earlier, the differences between different areas in Sweden with respect to the frequency of accessory chromosomes might not necessarily be the result of selective forces. It is, however, likely that selection under different ecologic conditions has at least played a part in the development of the present situation. This conclusion is supported by the indications of an influence of edaphic factors on the frequency of accessory chromosomes on the island of Öland, as well as by previous results from studies of accessory chromosomes in *Festuca pratensis* (BOSEMARK, 1956 a) and *Centaurea scabiosa* (FRÖST in MÜNTZING, 1954).

The cytological studies have revealed in practically all respects a striking similarity between the accessory chromosomes in *Phleum phleoides* and such chromosomes in other grasses like maize, rye, *Anthoxanthum*, and *Festuca*. This and studies of still other grasses with accessory chromosomes (BOSEMARK, unpublished), show that accessory chromosomes in the family *Gramineae* belong to a fairly homogeneous group, although in each species the behaviour and effects of the accessories vary in minor details.

BÖCHER (1950), on the basis of his studies, states that somatic non-disjunction of accessory chromosomes prior to meiosis has been ascertained in *Phleum phleoides*. In the present material no evidence of such a mechanism was obtained. Thus not only within different root-tips and flowers was the number of accessory chromosomes found to be constant, but the number counted in the root-tips always corresponded to that found in the flowers from one and the same plant. The results obtained by BÖCHER from a study of progeny plants from an evidently open pollinated mother plant with $2n=14+2$ acc. has no bearing on this question. Instead, the occurrence of non-disjunction of the accessory chromosomes at the first pollen mitosis has been clearly demonstrated. It is of special interest that the underlying mechanism has been found to be exactly the same as in rye, *Anthoxanthum* and *Festuca*. As in the two latter cases the mechanism is solely confined to the male side. With respect to the general effectiveness of the non-disjunction mechanism and the consequences of this on the maintenance and spreading of the accessory chromosomes in natural populations, the present observations are too few to justify definite statements. The high frequency of accessory chromosomes found in natural populations in Sweden and the results of the crosses, however, suggest that the non-disjunction

mechanism in *Phleum phleoides* is at least as effective in maintaining and spreading the accessory chromosomes as it is in *Festuca pratensis*.

SUMMARY

(1) The paper deals with cytology, transmission and frequency of accessory chromosomes in *Phleum phleoides*, a diploid, perennial grass species.

(2) Out of 1402 plants collected from most of the distribution range of the species in Sweden, 31,1 per cent had one or more accessory chromosomes, the average number of accessory chromosomes being 0,63. There is a marked difference between different areas with respect to the frequency of accessory chromosomes, but, as the distribution of *Phleum phleoides* in Sweden is discontinuous, these differences might be due solely to historical factors. Indications of an influence of ecologic factors on the distribution of accessory chromosomes on the island of Öland, however, suggest that such factors are at least partly responsible also for the differences between the different areas.

(3) The majority of accessory chromosomes in *Phleum phleoides* have a sub-terminal centromere, being about $^3/_5$ of the average of the normal chromosomes in length. In a few plants smaller and larger derivatives of the standard type have been found. Some of these are isochromosomes, others are the result of terminal deficiencies. The number of accessory chromosomes in the root-tips from one and the same plant has been found to be always constant and to correspond to that of the pollen mother cells.

(4) The meiotic behaviour of the accessory chromosomes largely corresponds to that of such chromosomes in other grass species.

(5) A mechanism of directed non-disjunction of the accessory chromosomes at the first pollen mitosis of the same type as that in rye, *Anthoxanthum* and *Festuca*, has been also found in *Phleum phleoides*.

(6) The studies of the inheritance of the accessory chromosomes have confirmed the cytological findings on the meiotic behaviour as well as the non-disjunction. The data indicate a high efficiency of the mechanisms for transmission and multiplication of the accessory chromosomes.

(7) The implications of the findings on the whole problem of accessory chromosomes in grasses are briefly discussed.

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CYTOGENETIC NOTES ON CHROMOSOMAL INTERCHANGES IN BARLEY

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I. INTRODUCTION

THE increasing use of chromosomal variants in crop plants as tools for attacking special cytogenetic problems of chromosome behavior, and also for direct or indirect applications to practical breeding makes it imperative that the available cytogenetic information on a given crop be summarized at frequent intervals. The notes reported here include break frequencies, notes on linkage tests, observations on changes in the somatic chromosomes, and selection of a set for testing linkage. They are the result of coordination of work at the Minnesota Agricultural Experiment Station with that at the Svalöf station in Sweden.

At both institutions a group of interchanges was isolated from irradiated material, the former in Mars, a six-rowed smooth-awned barley; and the latter in Gull, Maja and Bonus, all closely related, two-rowed, rough-awned varieties. It may be of some interest that the cross of Mars with Gull under certain conditions gives an F_1 with brittle rachis and dehiscent awns. As reported by WHITE *et al.* (1948) and BURNNHAM *et al.* (1954) the work with the Mars interchanges has led to the establishment of a tester set of interchanges, for use in inter-crosses to identify the chromosomes involved in other interchanges, tests for linkage with genetic markers in the various linkage groups, and for locating unlinked markers. Data for several of them have been published by HANSON and KRAMER (1949 and 1950), HANSON (1952), KRAMER, VEYL and HANSON (1953) and KRAMER (1954). Other linkage data will be summarized elsewhere.

The work at the Svalöf station has included the development of the oxyquinoline plus acetic orcein cytological technique for use in studying root-tip mitoses by which the different chromosomes of normal barley have been identified morphologically, TJIO and LEVAN (1950) and TJIO and HAGBERG (1951). The translocation lines isolated and studied at Svalöf are earlier described by NYBOM (1954 and 1956) and by HAGBERG (1954). The oxyquinoline technique was extended to these translocations

as well as to the Minnesota tester set and to other Minnesota translocations. It was demonstrated that in certain cases the exchanged pieces differed sufficiently to determine which chromosomes had exchanged pieces (HAGBERG and TJIO, 1950 and 1952, TJIO and HAGBERG, 1951).

Several new intercrosses between the Svalöf interchanges and the Minnesota tester set were made at the Washington Experiment Station at Pullman by HAGBERG, and grown and examined by him at the Minnesota Experiment Station.

II. BREAK FREQUENCIES, DESIGNATION OF CHROMOSOMES AND LINKAGE GROUPS

The interchanges that have been identified or partially identified as to the chromosomes involved are listed in Table 1, together with the frequencies of breaks in the different chromosomes. All the chromosomes have been involved, but in Mars, the frequency of breaks in chromosome *b* seems to be higher than for any other chromosome. In the two-rowed varieties the numbers are smaller but the breaks in *e* are low in frequency, with no marked differences between the others. In general, there seems to be no close relation to length. Also, in the entire group of 40 interchanges completely identified, only four of the 21 possible combinations are missing, *i. e. a—f*, *a—g*, *d—e*, and *e—g*. In recent studies three out of these four combinations were found in the two-rowed material. In the 27 Mars interchanges eight combinations were missing; but three of these were found in the 13 identified Svalöf interchanges. The limited data suggest a difference between Mars and the two-rowed varieties as to the frequencies of breaks in the *b* chromosome, and also in the frequencies of different combinations between chromosomes.

It is desirable to set up a usable uniform system of designating the chromosomes and the linkage groups which can be used until the correspondence between the various systems is established beyond doubt.

The linkage groups have been designated by the Roman numerals I to VII from the beginning of the genetic work on barley. Roman numerals have been used by TJIO and LEVAN (1950) and earlier by LEWITSKY (1931) (*c. f.* MECHELKE, 1955) for the chromosomes based on their morphology in root-tip divisions but it is proposed now that the Arabic notation be used for this purpose as shown in Table 2. It is used in the remainder of this report. The coordination of this Arabic notation with the letter designations based on the intercrosses between interchanges, and on their root-tip cytology is shown also in Table 2. OINUMA's letter designations are included in the table, and the BURNHAM *et al.*

TABLE 1. *List of interchanges in barley, including information on their cytology and on the break frequencies in each chromosome.*

TABLE 1. *List of interchanges in barley, including information on their cytology and on the break frequencies in each chromosome.*

	g	f	e	d	c	b	a	One chromosome identified	Total**		Grand*** total
									Mars	2-rowed	
a			C 1377 C 1456	'A'	C 1376 ≠ 1 C 1427 XT 11	C 1343 C 1384 C 1385 Ert 7 XT 3 XT 8 XT 12	—	XT 16 = a — e or f NT 6 = a — ?	7	5	15
b	C 1315 C 1376 ≠ 2 C 1462 C 1483	C 1310 K 9*	C 1346	C 1025 C 1317 C 1358 C 1363 C 1478	C 1365 K 8*	—			15	4	21
c	XT 13	C 1336 Ert 1 Ert 14 Ert 47 XT 4	C 1432 C 1434	C 1340 C 1405	—			C 1440 = c — f or g C 1449 = c — e? 'B' = c — b or d	8	6	18
d	XT 2 XT 17	XT 15		—				XT 5 = d — ? NT 9 = d — ?	7	3	13
e		C 1420 C 1482	—						7	0	7
f	C 1433	—							5	5	11
g	—							XT 1 = g — a or c XT 14 = g — c or d	5	3	10
								Total sum of breaks:	54	26	95

No underline =karyotype normal
Underlined ——— = » easily distinguishable
» — — — = » distinguishable with some difficulty
» ..... =no root-tip cytology

* K 8 and K 9, KRAMER's interchanges.
** Totals include only those completely identified in these varieties.
*** Grand totals include those completely or with one chromosome identified from all sources.

TABLE 2. *Summary of the correspondence of various systems of designating chromosomes and linkage groups in barley.*

Reference	Chromosomes							Based on
OINUMA, 1952	a	c	d	e	g	f	b	root-tip cytology
LEWITSKY, 1931	III	IV	VI	V	VII	I	II	»
TJO and LEVAN, 1950	I	II	III	IV	V	VI	VII	»
proposed:	1	2	3	4	5	6	7	
BURNHAM *et al.*, 1954	f	b	c	e	a	g	d	interchange intercross
		III						
ROBERTSON *et al.*, 1955 ..	I	+ VII	VI	IV	II?	—	V?	linkage data

letters are the ones referred to in the discussion that follows. The three longest chromosomes include *f*, *b* and *c*. Although not established with certainty, the indicated correspondence is believed to be the most probable. When finally established, the Arabic system of 1 to 7 will be sufficient for the chromosomes and the linkage groups. Then the interchanges can be designated in the same manner as those in maize, *i. e.* 1—2, 1—3 *etc.* using *a*, *b*, *c* to designate different ones involving the same chromosomes.

The correspondence between the linkage groups and the letter designations of the chromosomes as used by BURNHAM *et al.* (1948 and 1954) is based on the published linkage tests between genetic markers and the interchanges (in addition to the references on page 467 see RAMAGE, 1955) and on unpublished data of WHITE, LIVERS, RAMAGE and BURNHAM.

KRAMER, VEYL and HANSON (1953) concluded that linkage groups III and VII are carried by the same chromosome and that the other established linkage groups belong to different chromosomes. Using positive linkages with the interchanges as a test (BURNHAM, in press) their conclusion regarding III and VII is confirmed. If the others are independent, the correspondence between them and the chromosomes as designated by the letters is that shown in Table 2. In three cases, however, linkage between only one interchange and one or more markers in that group has been found, *i. e.*, C 1432 (*e—f*) showed linkage with V in group I, C 1456 (*a—e*) was linked with B in group II and C 1478 (*b—d*) gave 24 % recombination with R and 12 % with S. Thus there is no evidence from these facts against group I and also II being in the *e* chromosome which has linkage group IV; also no evidence against group V being carried by *b* which has groups III and VII. Linkage data involving C 1432 and factors in groups I and IV indicate that these two groups are

independent (RAMAGE, 1955). This leaves the possibility that chromosomes *a* and *d* have no known genetic markers. As shown in Table 2, it is certain that for *g* no genes have thus far been found. It is proposed that *g* be used as the temporary designation of this linkage group. Interchanges are being used to establish this linkage group; a more efficient selection being possible now based on information from root-tip cytology (see page 475) and from stocks to be selected from the linkage tests (BURNHAM, in press).

III. NOTES ON THE USE OF INTERCHANGES IN LINKAGE STUDIES

A few notes on linkage tests based on the experience of the senior writer may be useful to others. In order to determine the interchange break point in a given linkage group, tests with two or more well-separated genes are ordinarily sufficient for material with normal chromosomes even in separate tests. With interchanges, however, if there are separate tests with each marker the position of the break point relative to the markers may still be unknown. This results from the reduction in crossing over caused by the heterozygous interchange, the degree not being known unless both linked markers are in the same cross. The greatest reduction is in the region between the break and the centromere in a species such as barley which has a high proportion of zigzag configurations (directed segregation) (HAGBERG, 1954, page 487). As KRAMER and HANSON (1949) have shown, the pattern of this reduction found in data involving interchanges with break points at different loci in the linkage group may give a clue as to the position of the centromere. The same has been suggested by LAMM (1949). More stocks with several markers in the same linkage group are needed. A concerted effort to produce usable multiple marker stocks for each chromosome is a necessity for the interchange studies.

For linkage tests, the most efficient method is to use homozygous interchange stocks for crosses with the gene markers. If a stock heterozygous for the interchange is used for crossing, the partially sterile plants (about 25 % pollen abortion) must be selected as one parent. Also the F_1 plants must be classified for partial sterility. Under good growing conditions where the plants are vigorous and well-tillered, classification for fertile vs. partially sterile plants based on seed set is satisfactory. Under poor growing conditions, *e. g.* late planting in Minnesota, pollen abortion is a more reliable criterion but still not infallible. Many of the recessive markers that are not lethal are weaker than normal, and both

head and pollen classification are often unsatisfactory. Barley seems to be extremely sensitive in this respect, in marked contrast to maize.

The solution of the difficulty in barley is to resort to the testing of F_3 lines. For seedling characters, whether lethal or not tests in F_3 can be made in the greenhouse during the winter months. This test determines the genotypes, (whether homozygous or heterozygous) of the partially sterile and fertile F_2 plants. If grown to maturity the F_3 may be used as a check on sterility classification as well. With linkage, the ratio in F_3 of partially sterile : fertile plants in the dominant class should deviate from 1 : 1 toward the 2 : 1 ratio expected for complete linkage. Since other factors, *e. g.*, gametophyte factors, may cause similar deviations, it is necessary to check the results in F_3. One case was encountered in which an apparent close linkage in F_3 was not confirmed in F_3.

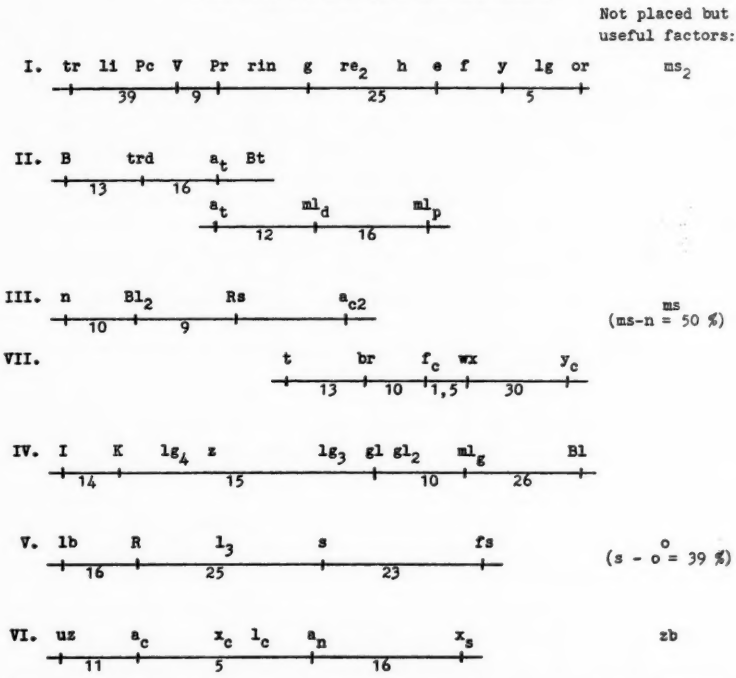
IV. BARLEY LINKAGE MAPS AND A LIST OF GENE SYMBOLS AND THE CORRESPONDING CHARACTERS

To have the genetic and cytological information in one place, the linkage maps are reproduced here in Table 3. These follow those given by ROBERTSON, WIEBE and SHANDS (1955) with minor changes. Characters commonly referred to by their dominant alleles are given capital letters. The recombination values between certain genes have been added for use as a guide in planning linkage tests.

The writers have attempted to rate the characters according to the following scheme:

1. Endosperm characters (only two known in barley, blue aleurone and waxy).
2. Seedling characters with viability and vigour equal to that of normal plants.
3. Seedling characters, not lethal but lower in viability.
4. Lethal seedling characters. Crosses must be made with heterozygotes. Unless balanced lethal stocks are available, more than one cross must be made to ensure including a heterozygote.
5. Adult plant characters that are as vigorous as normals.
6. Same as 5, but lower fertility. Included here is male sterility. Male sterile plants cannot be classified for interchange sterility. They can be used as the female parents in crosses.
7. Adult plant characters with lowered viability.
8. Relatively unusable characters due to poor expression or complex inheritance.

TABLE 3. *Linkage groups in barley. Recombination values reported between certain loci are included.*


Not placed but useful factors:
I. tr li Pc V Pr rin g re2 h e f y lg or
39 9 25 5
ms2
II. B trd at Bt
13 16
at mld mlp
12 16
III. n Bl2 Rs ac2
10 9
ms
(ms-n = 50 %)
VII. t br fc wx yc
13 10 1,5 30
IV. I K lg4 z lg3 gl gl2 mlg Bl
14 15 10 26
V. lb R l3 s fs
16 25 23
o
(s - o = 39 %)
VI. uz ac xc lc an xs
11 5 16
zb

In all but class four, crosses with the homozygous markers are possible. The endosperm characters have the one advantage that the genotype determinations based on F_2 may be made by examining the seed produced by F_2 plants. For classes 2, 3 and 4, F_2 tests in the greenhouse are usually possible and should be used.

Adult plant characters require that the F_2 tests be run in the field. Not only does this require extensive field space, but disease attacks may interfere. In general, seedling characters are easier to use than adult characters, unless sterility is to be checked also in F_2. The classes which include the crossovers should be grown in F_2 to maturity to check the character and the sterility classification.

An interchange may be present already in the gene marker stock. Probably the best method of detecting this is to study meiosis in the F_1 plants. It might be indicated also by higher degrees of sterility as well as a lower proportion of fertile plants in F_2.

The gene symbols in the genetic maps are listed alphabetically, together with the characters they represent. The first number following the symbol is the linkage group to which it belongs, the second is the rating described above.

Gene symbol	Linkage group	Rating	Description of characters
a		4	albino seedling: a_c in group VI, from Colsess. a_{c2} in group III, from Coast. a_t in group II, from Trebi
B	II	5	black lemma and pericarp
Bl	IV	1	one of two factors complementary for blue aleurone
Bl_2	III	1	» » » » » » » »
br	VII	1,5	brachytic, short internodes in stem and rachis (variable), rounded leaf-tip in seedling
Bt		5	one of three factors complementary for brittle rachis
e*	I	5	elongated, (long-awned glumes)
f	I	3	chlorina seedling; f_c in group VII, from Colsess V
fs	V	5	fragile stem
G	I	8	teeth on lemma
gl, gl_2	IV	3	glossy seedling
gp	?	5	grandpa — white head and upper leaf and sheath, sometimes white striped leaves
H	I	8	height
I	IV	5	infertile lateral florets (classification may be difficult)
K	IV	5	hooded awns
l	V	5(8?)	dense spike (club) l_3 in group V; two of at least 7 factors (l=lax)
lb	V	8?	long basal internode
lc	III	6	» chromosomes (about 20 % sterility)
lg	I	3	light green seedling, lg_3 and lg_4 in group IV
li	I	2	liguleless
ml		2	susceptibility to mildew, two factors in II, one in IV
ms	III	6	male sterile; ms_2 in group I
n**	III	5	naked caryopsis, palea (hull) not adherent
o	V	5	orange lemma
or	I	3	» seedling
Pc	I	5	purple-veined lemma
Pr	I	5	purple straw
R	V	5	main factor for rough awns
Re_2	I	5	red pericarp, one of two complementary factors
rin	I	5(8?)	dense spike (rachis internode number)
Rs	III	5	red stem (classification may be difficult)
s	V	5	short-haired rachilla
t	VII	2	susceptibility to stem rust, *P. graminis tritici*
tr*	I	5	triple-awned lemma
trd	I	5?	third outer glume
uz	VI	3?	uzu, semi-brachytic
V	I	5	two-rowed ears

Gene symbol	Linkage group	Rating	Description of characters
wx	VII	1	waxy endosperm, starch stains red-brown with iodine, also in pollen***
x		4	xantha (yellow seedling); x_c and x_s in group VI
y	I	4	virescent, y_c in group VII
z	IV	2?	zoned (pale green cross-bands) on leaves
zb	VI	2	zebra; white cross-banding similar to albescent in maize but in barley it is expressed only in seedlings grown in early field plantings (cool nights?)

* The combination of *tr* with *e* is relatively infertile.

** *n*, hull-less Nepal, Himalaya and Brachytic No. 119 give poor seed-sets when used as females in crosses, *N* Nigrinudum and Nudideficiens are usable as females.

*** Classification in the pollen made easily by staining with iodine — KI solution, using no light from below the stage, side lighting from above.

V. BREAK POSITIONS IN THE INTERCHANGES

One goal of these studies has been to establish interchanges that mark the arms of the different chromosomes. These can be used in more efficient linkage tests for testing the independence of the linkage groups, locating unlinked characters, and possibly in the study of economic characters.

The information that follows is based for the most part on observations by HAGBERG using the root-tip squash technique as reported by TJIO and LEVAN (1950). All of the homozygous Minnesota interchange stocks, including the tester set were examined as well as all the Svalöf translocations. Earlier observations on the Minnesota tester set of interchanges have been published (HAGBERG and TJIO, 1952), also for the translocation linked to *Erectoides* 7 (HAGBERG and TJIO, 1950).

The results reported here are more of preliminary type but may serve as a guide for future work. No measurements were made. For those in which large differences were found there is little doubt as to the morphological chromosomes involved. Indications of smaller changes were found also. Where they were consistent in several good plates, they are reported; others that seemed to be present are reported also, the doubt being indicated. Detailed study of root-tip chromosomes, using photographs and measurements combined with pachytene study, should disclose further differences. Preliminary work on pachytene stages indicates that some information can be gained from that source.

A change in one of the shorter chromosomes or in either of the satellited chromosomes is easier to recognize than a change in a longer chromosome. For the observation that a certain chromosome seemed to

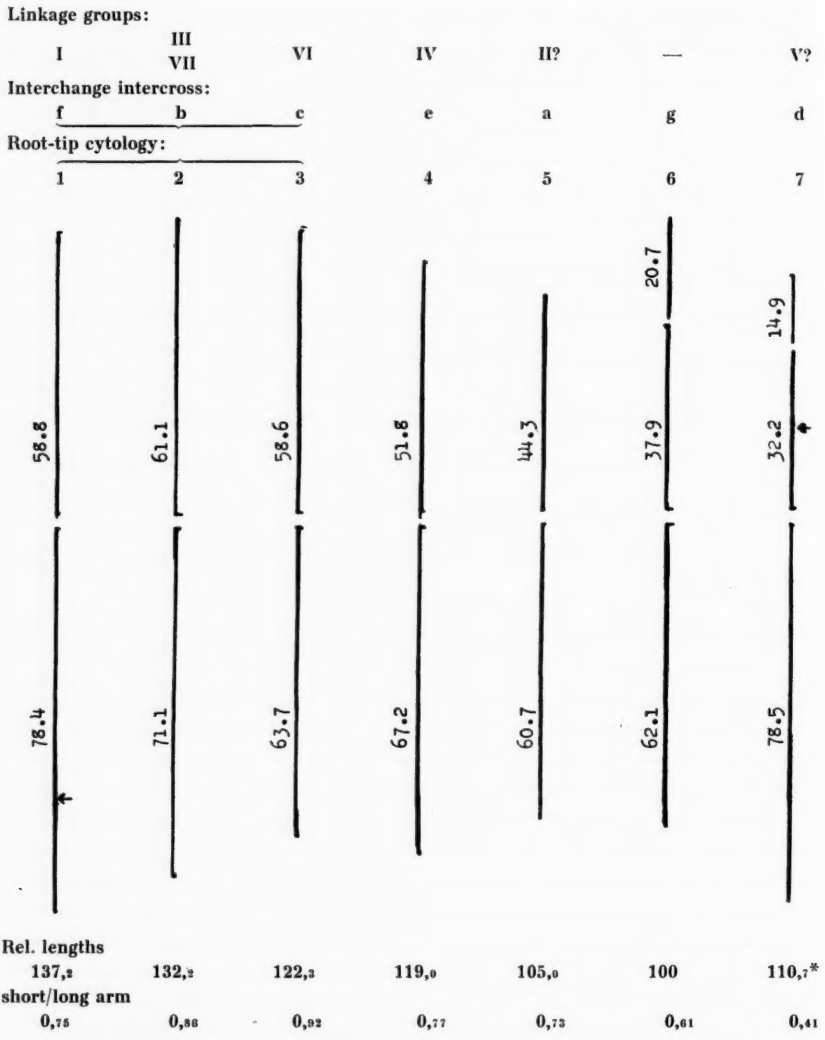


Fig. 1. Morphology of the 7 chromosomes in barley, based on root-tip cytology and correlation of linkage groups and systems of designation.

*** Calculated from relative lengths and indices in descriptions by TJIO and HAGBERG (1951). The arrows indicate position of »tertiary» constrictions observed occasionally (see page 478).**

have one arm shorter or longer than normal there may sometimes be more than one interpretation possible of the position of the breaks. It may be difficult or impossible to determine on which side of the centromere they are localized. The two possibilities cannot be distinguished by root-tip cytology only.

For the interchanges involving *d* or *g*, but with no change observable cytologically, the most probable position is in the long arm, although there may have been an exchange of small and equal pieces with one of the breaks in the satellite. As discussed above there is the possibility of breaks in the short arms equidistant from the centromeres sometimes giving an apparently unchanged chromosome.

The results are in alphabetic order according to the chromosomes involved in each interchange.

a—b	Ert 7	Short arm of 5 roughly half its normal length; long arm of b longer than normal (HAGBERG and TJIO, 1950)
	C 1343	Chromosome 5 has a median centromere, the short arm having gained a longer piece; short arm of b shorter than normal
	C 1384	Long arm of 5 longer than normal
	C 1385	No obvious change
	XT 3	» » »
	XT 8	» » »
	XT 12	Long arm of 5 possibly longer than normal and one chromosome subterminal
a—c	C 1376≠1	No obvious change
	C 1427	Long arm of 5 longer than normal
	XT 11	No obvious change
a—d	'A', Smith	» » »
a—e	C 1377	Long arm of 5 shorter than normal, centromere almost median
	C 1456	No obvious change
a—e or a—f	XT 16	Long arm of 5 very short
a—?	NT 6	» » » » » »
b—c	C 1365	Short arm of one of the long chromosomes shorter than normal
b—d	C 1025	Satellite of 7 too long; no obvious change in any other chromosome
	C 1317	Long arm of 7 changed?
	C 1358	Short arm of 7 (excluding the satellite) somewhat longer than normal; the break in 7 was in the short arm between the »tertiary» constriction and the nucleolar organizer region (secondary constriction); the long arm of one of the long chromosomes now has the satellite from 7

A »tertiary» constriction in the short arm of 7 shows up occasionally, see Fig. 1, compare HAGBERG and TJIO, 1952 and MECHELKE, 1955. In the earlier report (HAGBERG and TJIO, 1952) the long chromosome was believed to be 3. In the report on the ert 7 interchange (HAGBERG and TJIO, 1950), chromosomes 1 and 5 were shown to be involved. At that time it was not known that ert 7 is an a—b interchange; one chromosome therefore being common to both it and C 1358. We do not know which of the long chromosomes is b but it is probably not 3.

b—d	C 1363	Break in 7 in short arm distal to the tertiary constriction
	C 1478	No obvious change
b—e	C 1346	» » »
b—f	C 1310	Two long pairs with median centromere, one being longer than 3. Since normal material shows only one long median chromosome, number 3, the exchange must have been between 1 and 2. If 2 is b then 1 is probably f (see also XT 15=f—d)
b—g	C 1315	No obvious change in g or 6, the chromosome with the long satellite
	C 1376≠2	Chromosome 6 seems to be short, the change being in the long arm
	C 1462	Long arm of 6 possibly too short
	C 1483	Chromosome 6 has a longer satellite, roughly twice as long as in normal stocks; one of the longer chromosomes has a short arm that is shorter by a similar amount (HAGBERG and TJIO, 1952)
c—d	C 1340	No obvious change in 7
	C 1405	» » » » »
c—e	C 1432	» large change, but two pairs seemed different
	C 1434	» obvious change
c—f	C 1336	Not examined
	XT 4	Has two short pairs without satellites
	Ert 1	No obvious change
	Ert 14	» » »
	Ert 47	» » »
c—g	XT 13	Long arm of 6 is probably too long
d—f	XT 15	» » » 7 was shorter than normal; short arm of one of the longer chromosomes was longer than normal
d—g	XT 2	One of the new chromosomes has both satellites, one at each end. The break in 7 was not distal to the tertiary constriction in the short arm. Since this new chromosome with the satellites is short and has a nearly median centromere, both breaks must have been near the centromeres, one in the long arm, the other in the short: either 6 S and 7 L or 6 L and 7 S
	XT 17	Probably each break in the short arm, the satellite ends having exchanged positions.

e—f	C 1420	No large change, but one pair seems to be different (subterminal centromere)
	C 1482	Not examined
f—g	C 1433	Long arm of 6 possibly too long

The above sets of information are summarized below. An attempt is made to select a group of interchanges which might be used as markers for the different arms of the seven chromosomes, these lines being indicated by an asterisk *. This set of markers is at the present time, of necessity, incomplete, but this information should be useful in planning studies needed to fill the gaps. If the proper ones are available, 7 would be needed to furnish one test with each arm. Supplemental ones would be needed, since each interchange marks two different arms. However, this goal is impossible at present for some of the chromosomes. In the summary below S means that the short arm is broken, L the long arm and Sat means that the break has occurred in the satellite. Those underlined are the ones most definitely established. An asterisk * marks those which have been selected as markers for this particular chromosome.

Chromosome a, number 5		Arm with observed change 1st chrom.	2nd chrom.
*Ert 7	a—b	*S*	*L*
C 1343	a—b	*S*	S
*C 1377	a—e	L	—
C 1384	a—b	L	—
C 1427	a—c	L	—
XT 12	a—?	L	—
XT 16	a—e or f	*L*	—
NT 6	a—?	*L*	—
Chromosome b, one of the longest			
C 1025	b—d	—	*Sat*
C 1343	b—a	S	*S*
C 1365	b—c	S	—
*C 1483	b—g	*S*	*Sat*
*C 1358	b—d	*L*	*S*
*Ert 7	b—a	*L*	*S*
C 1384	b—a	—	L
C 1310	b—f	—	—

Accurate measurements might disclose a change in *b* in C 1384, a—b; C 1363, b—d; C 1376-2, b—g; and C 1462, b—g. The change in C 1025, b—d, although observable in *d*, is probably too small to be detected in *b* even with measurements.

Chromosome c, probably number 3 with median centromere			
XT 4	c—f	L?	—

C 1405, c—d, shows close linkage with x_c. Detailed study and measurements of the most favorable ones are needed. An observable change might be expected in C 1365, b—c; C 1427, a—c; C 1432, c—e; XT 13, c—g; and 'B', c—b or c—d.

Chromosome d, number 7		Arm with observed change 1st chrom.	2nd chrom.
*C 1358	d—b	S	L
*C 1025	d—b	Sat	—
C 1363	d—b	S	—
XT 17	d—g	S?	S?
XT 2	d—g	L	S
		S	L
NT 9	d—?	S	L
*XT 15	d—f	L	S

From linkage tests there is the information that one of the break points in line C 1478, d—b is probably in the long arm of chromosome *d*, if linkage group V is on *d*.

Chromosome e, probably number 4.

Those favorable for detailed study are: C 1377, e—a; XT 16 (if e—a), and possibly C 1420, e—f; and C 1432, c—e. According to HANSON and KRAMER (1949) the linkage data suggest the break in *e* in C 1420 and C 1432 is close to the centromere.

Chromosome f, one of the longest chromosomes

XT 15	f—d	S	L
XT 4	f—c	—	L?

Those favorable for detailed study are: C 1310, f—b; C 1433, f—g; and possibly C 1420, f—e.

Chromosome g, number 6

*C 1483	g—b	Sat	S
XT 17	g—d	S?	S?
*C1376+2	g—b	L	—
C 1433	g—f	L	—
C 1462	g—b	L	—
XT 13	g—c	L	—

SUMMARY

The break frequencies for the seven chromosomes of barley in 27 interchanges in the Mars variety suggests a higher frequency in the *b* chromosome than in the others. This was not true of the thirteen interchanges identified in two-rowed varieties. The variety groups seem to

differ also in the frequencies of the different chromosome combinations. All but four of the 21 different combinations possible have been found.

The Arabic notation 1 to 7 for the chromosomes is proposed in place of the Roman numerals used by TJIO and LEVAN (1950). The correlation thus far determined between these, the letter designations which have been used and the linkage groups are summarized. Notes on the use of interchanges in linkage tests in barley, and a reprinting of the linkage maps are included.

Based on cytological information from root tip sqashes, at least one interchange marking each chromosome arm and the satellites is available for chromosomes *a*, *b*, *d*, and *g*. For *c* and *f* there is a probable marker for only one arm each. Interchanges involving, *c*, *f* and *e* should be studied to identify the ones usable as markers.

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STUDIES ON INDUCED MUTATIONS IN MALE AND FEMALE GERM LINE OF DROSOPHILA MELANOGASTER

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IT has been known for many years that there can be induced relatively high rates of yellow mutations in inverted X-chromosomes where the proximal heterochromatin is closely connected to the distal »y ac sc» region, (for ref. see LÜNING, 1952). In recent years I have made use of this in a number of experiments. Most of these have been confined to studies on the rates of mutations induced in X-chromosomes carried in sperm. There was, however, also one group of experiments where oocytes were irradiated as well, LÜNING (1954). In this paper it was stated that there was induced a higher rate of yellow mutations in the MULLER-5 (sc^{S1} *B InS* w^a sc^8=M5) chromosome in oocytes than by the same dose in sperm of 0—1 day old males ready the first 2 days after irradiation. In this study I made no difference between variegated and non-variegated yellow mutations, all were simply classed as changes in the yellow region. There seemed, however, to be a slight predominance of the variegated cases after irradiation of oocytes. To test this further, the following small experiment was performed.

The purpose of the experiment was also to study another locus, *viz.* *w*, in which variegated as well as non-variegated mutations are known to appear. To study both yellow and *w* mutations, a modified MULLER-5 chromosome (sc^{S1} *InS* w^x sc^8 ♀×sc^{S1} *InS* w^x sc^8; *y* sc^8 *Y*) was produced. To facilitate the detection of eye-colour mutations, Bar was excluded and instead of w^a another allele w^x was used, which gave a colour close to wild type. This w^x allele was obtained as a mutation from the w^a allele in a previous experiment. — This modified MULLER-5 chromosome will later on be called M5*rx* (*r*=round, *x*=dark *w*-allele). The *y* sc^8 *Y* chromosome was used to detect spontaneous yellow mutations in the stock, as described by LÜNING (1954 a). 4 days old virgin M5*rx* females and 0—24 hours old M5*rx*; *y* sc^8 *Y* males were mixed immediately prior to the irradiation with 3240 r, and were then separated and mated to *y w spl sn*; sc^8 *Y* males and *y w spl sn* females, respectively. The irradiated

M5*rx* males were transferred to new virgin females after 24 hours and were then discarded after another 24 hours. The irradiated M5*rx* females were allowed to lay eggs in vials for 2 days and were then transferred to new fresh vials. They were shifted 3 times, in that way giving four batches of eggs. In this series the vials were numbered, which made it possible to detect clusters of mutations. There was detected one cluster of *w*-mutations (3 ♀♀) which proved to be lethal in the progeny test; these were excluded from the material in Table 1.

TABLE 1. *Induction of yellow, fractional yellow, variegated yellow, white, coloured white, white split, white split Notch and Minute mutations in sperm or oocytes from the stock M5rx* (sc^{S1} *InS* w^x sc^8) *tested against females and males respectively of the y w spl sn stock. Sperm was from matings the 1st and 2nd day after irradiation. Eggs were collected from irradiated females during 4 2-day periods.*

Ser.	S:a	y	$t^{r \cdot y}$	y^{var}	w	w^x	w spl	w spl N	Mi
♂ 1	5510	13	3					1	56
♂ 2	11763	26	6		5	1			70
♂ Tot	17273	39	9		5	1		1	126
♀ 1—2	8904	20		13	3	2	1	1	258
♀ 3—4	10768	22	1	11	6		3	1	255
♀ 5—6	16119	40		9	7		1	3	346
♀ 7—8	13753	20		8	3		1	2	198
♀ Tot	49544	102	1	41	19	2	6	7	1057

The yellow mutations have been classified in four classes according to their being full or fractional (mosaics), variegated or non-variegated mutations. Here it ought to be pointed out that this type of variegated mutation is basically yellow in appearance but with intermingled grey areas over the whole body. In the normal M5 stock there can appear a certain variegation where the basic colour is wild type with small yellow patches (LÜNING, 1954 b). The penetrance of this type is mostly low but depends on modifiers (BRUNSTRÖM, unpublished). The variegation studied in the present investigation has 100 % penetrance as will be further discussed below.

Mutations in the *w*-region were classified as for the variegation or non-variegation, and, *spl* and *N* showed together with *w*. Mutations in *spl* and *N* loci were also recorded separately but shown by progeny tests to be uncertain. Cases appeared which were first classified as *spl* but

turned out to be dominant mutants of rough type. The results are presented in Table 1.

From this it is seen that non-variegated full yellow mutations are induced in about the same frequency in sperm and oocytes with a tendency in the latter to decrease with time from irradiation; this means a lower frequency in earlier stages. As regards variegated full yellow mutations it is seen that in the present test all are obtained after irradiation of oocytes. From earlier tests (LÜNING, unpublished) it is known that variegated yellow mutations appear in a very low frequency after irradiation of sperm. On the other hand fractional mutations appear in a higher frequency after irradiation of sperm than oocytes. This is, however, not unexpected as sperm has passed meiosis and might contain chromosomes already split into chromatids which will separate in the first cleavage division of the zygote, while the oocytes contain chromosomes whose chromatids will separate and be included in polar body nuclei and egg nucleus.

If we then compare the *w*-mutations in the male and female irradiation series it is seen that there are induced about the same rates of *w*, *w spl* and *w spl N* in both. There is no tendency to a high rate of *w*-variegations at irradiation of oocytes. Hence there does not seem to be a universally higher rate of variegated mutations in oocytes than in sperm. The difference obtained in the case of yellow might be very specific. In any case it shows that the mutation spectrum also within very short regions of a chromosome might differ considerably between the two sexes.

To obtain further information about differences and similarities a number of the mutations were progeny tested. Out of 34 non-variegated yellow mutations from irradiated oocytes all were in addition both achaete and lethal. Of 18 tested variegated yellow mutations all gave variegated offspring, which as far as could be seen were also *ac*-variegated; all but one were lethals. The single fractional yellow in the »female series» was a gonad mosaic with both y^+ and *y* males vital. From the male series 12 yellow mutations were tested, all were also achaete and lethals.

From other studies on this type of yellow-mutations (STRÖMNÆS and LÜNING; unpubl.) it is known that parts of the lethals can be covered by the sc^8 *Y*, which contains a small segment of the *y ac* region from an X-chromosome. Cases which are lethal with the normal *Y* but viable with sc^8 *Y* must contain the locus or loci with lethal action within the small region which is translocated to the sc^8 *Y* chromosome. In order to

test the ability of the sc^8 *Y* chromosome to cover the lethals induced in oocytes a special test was made for this. Of 28 non-variegated yellow mutations only 2 were not covered by sc^8 *Y*, and of the 18 variegated yellow mutations all were covered. These results show that both variegated and non-variegated mutations are confined to changes in the same small region and that the differences obtained cannot be further studied with the present methods.

Among the *w*, *w spl* and *w spl N* mutations 12 were tested from the female series. All but one were lethals.

Beside these mutations in specific loci or regions of the X-chromosome there was also recorded another type of mutations affecting bristles — Minutes. This type of mutation had recently been used by GLASS (1952) in a comparative study between x-rayed sperm and oocytes. In agreement with GLASS's figures there is a predominance of Minutes in the offspring from irradiated females over that from irradiated males.

The rates of the three types of aberrations induced indicate that the frequencies in oocytes are *at least* as high as in mature sperm. This is contrary to the reports on dominant lethals. A study of this discrepancy is under way and the problem will be further discussed when these data are available.

CONCLUSIONS

A comparison of the mutagenic effect of x-rays on mature sperm and oocytes shows that for two types of mutations, *viz.* fractionals and variegated-yellow mutations, there is a difference. In sperm there is induced a rather high rate of fractionals while these are rare in oocytes. On the other hand there is induced a considerable frequency of variegated yellow-mutations in oocytes whereas that type is very rarely induced in sperm. Another locus known »frequently» to mutate to variegated-states *viz.* white, did not show such a predominance in irradiated oocytes. It is concluded that there exist differences between the mutation spectrum and that this might be confined to small regions.

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COMPARISON OF THE EFFECTS OF 8-ETHOXYCAFFEINE AND X-RAYS ON THE CYTOLOGY AND GROWTH OF ROOTS OF VICIA FABA

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I. INTRODUCTION

IT would be an obvious gain if more were known regarding the chain of events which occurs between the absorption of ionizing radiation by a tumor subjected to radiotherapy, and the regression of the tumor. It is natural to speculate whether the overall clinical effect observed stems from a certain specific injury to the cell, and whether only a particular cell structure is involved. An attempt has been made to answer this question over numerous years by a study of the responses of the root of the broad bean, *Vicia faba*, to various radiations under a variety of conditions. The obvious difference between tumor tissue and this root gave the earlier experiments a tentative and exploratory nature. The first major study was on the influence of the linear ion density characteristic of different radiations on the response of the root, and it was gratifying that accumulating results of similar type observed when normal and malignant animal tissues (including the human) were irradiated showed the same characteristics. This gave encouragement for further experiments on the root, and it was found that in regard to the inhibition of mitosis, the influence of dose rate, the influence of dissolved oxygen, the fragmentation of chromosomes, the promotion of cell differentiation, and the permanent suppression of cell division, there were also striking similarities with observations on one or another animal tissue including, in some cases, those of cancer patients. Although this is no guarantee that future conclusions based on a study of the bean root will be applicable to human tissue, the general consistency of the agreement in behavior so far makes this probable. The experimental approach has been to study how the sensitivity of the roots is altered by changes in their environment while being irradiated, or in some cases before or after irradiation. This sensitivity has been measured on the one

hand by the reduction in growth rate or in growth of the roots (corresponding to the regression of a tumor after irradiation) and on the other hand by cytological and histological studies to try to find some microscopic change which always varies in the same way as the gross effect on the growth. The results which had accumulated up to 1951 enabled GRAY and SCHOLES (1951) to list five cases in which the macroscopic change in response of the roots had a close parallel in the change in the proportion of metaphase or anaphase cells which showed chromosome aberrations. Subsequently GRAY (1954) found that the macroscopic X-ray sensitivity of the roots was altered by carbon monoxide-oxygen mixtures in a parallel manner to the alterations in chromosome damage observed in *Tradescantia* microspores by KING, SCHNEIDERMAN, and SAX (1952).

In recent years KIHLMAN has described the induction of chromosome aberrations in plant root cells by solutions of purine derivatives and in particular by 8-ethoxycaffeine, which will be denoted below by EOC. The publication most relevant to this work, since it deals chiefly with *Vicia faba*, is that of KIHLMAN (1955 b). There are some striking similarities between cytological observations made on roots which have been irradiated and those subjected to treatment with EOC. In particular very few, if any, aberrations are produced if the solution is devoid of oxygen. When the oxygen concentration is raised, the numbers of aberrations produced by a fixed treatment increase in a way which could, at first sight, be mistaken for the well known increase observed when X-rays cause the aberrations. However, the potency of an EOC solution, as judged by its capacity to produce chromosome aberrations, can be altered by a variety of environmental changes which do not affect the production of aberrations by X-rays. Our ability to alter the production of aberrations in these ways enables us to test, whether the parallel between growth of the roots and the degree of chromosome damage which has been observed when the radiations were used, will also exist when EOC is the cause of the damage.

II. COMPARISON OF THE MODIFYING EFFECT OF ENVIRONMENTAL CHANGES ON THE REDUCTION OF GROWTH AND THE PRODUCTION OF CHROMOSOME ABERRATIONS BY EOC

The methods used by KIHLMAN in the cytological study have been described in a number of papers (KIHLMAN, 1951, 1952, 1955 a, 1955 b).

The culture of *Vicia faba* roots, their measurement and the method of handling the data have been described by GRAY and SCHOLES (*loc. cit.*) and by READ (1952). An EOC treatment of roots for the growth study was given as follows. The perspex cell used for the X-irradiation of roots, illustrated in Fig. 2, READ (1952), was filled with an EOC solution saturated at room temperature, giving a concentration of 7×10^{-3} M. The cell was immersed in a sink of water to which ice was added at intervals. It was not difficult to keep the temperature of the solution close to 10° C, a variation of 2° C being very unusual. Air was bubbled through the cell or, if an anaerobic treatment was desired, nitrogen. In one experiment a number of different air-nitrogen mixtures were used. When conditions had reached the desired state, a group of 9 roots was immersed in the solution for periods ranging from 2 to 4 hours. Immediately after removal they were immersed in running tap water and returned to the culture tank. The mean growth made by the group of 9 roots in the next 10 days, compared with the group of an untreated control group was the measure of effect used in the experiments of this section. KIHLMAN's cytological observations are given in Fig. 1 A and in Tables 1 A to 5 A and the corresponding growth measurements are given in Fig.1 B and in Tables 1 B to 5 B. Each table distingushed by a B refers to a treatment very similar to that applicable to the same number with an A.

When the EOC solution contained no oxygen almost no aberrations were observed whereas a normally aerated solution produced a large number (1 A). This result is typical of a number of observations. Table 1 B shows that the aerated treatment produced a marked reduction in growth. By »Equivalent X-ray dose« is meant the dose of X-rays of 1 mm Cu H. V. L. which, when given to the roots in normally aerated water, and in a period of 10 minutes, produces the same reduction. When no oxygen was present, there was virtually no effect. Four other such pairs have been obtained for shorter treatment times but these were done with solutions at 19° C, instead of at 10° C. KIHLMAN (1951 and unpublished) has found that in addition to the effect on the chromosomes, there are two different toxic effects of EOC, resulting in the death of the roots. (See Discussion, page 016.) Both toxic effects increase with increase in temperature. On the other hand, chromosome aberrations are maximum when the temperature is at 10° C. In the growth experiments at 19° C with aerated solutions, a considerable number of roots were killed as a result of the toxic effect of EOC and the growth of the remainder was markedly reduced. If the EOC solution was with-

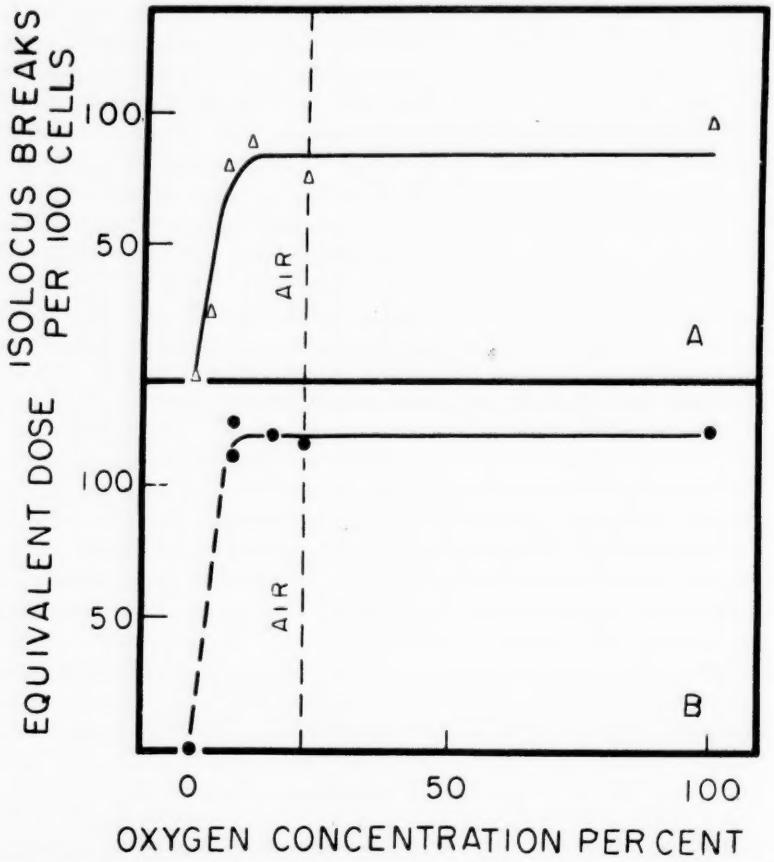

Fig. 1. The influence of oxygen concentration on (A) the yield of isolocus breaks (data from KIHLMAN, Hereditas 41: 384, 1955) and (B) the reduction in growth obtained after treatments with EOC.

out oxygen, no roots died but there was a small reduction in growth as shown in 3 B. All the other growth experiments which were done subsequently, were done with 10° C solutions to minimize the toxic effect.

KIHLMAN has also studied the cytological effect of 4-hour treatments with EOC at oxygen concentrations other than 0 and 21 per cent (air), namely, 2,7, 5,7, 10,6, and 99,7 per cent (KIHLMAN, 1955 b). These experiments were carried out at 10° C. The results are shown in Fig. 1 A,

TABLE 1 A. *The effect of anoxia during 4 hour treatments with 10^{-2} M EOC at 10° C on the production of chromosome aberrations.*

Treatment conditions	Recovery period hours	Number of methaphases analyzed	Abnormal methaphases per cent	Aberrations per 100 cells	
				Isolocus breaks	Exchanges
Normal aeration	19	100	64	79	22
No oxygen	19	200	2	1	0,5

TABLE 2 A. *The effect of a 2 hour period of anoxia immediately before a 2 hour treatment with normally aerated 10^{-2} M EOC at 10° C.*

No pretreatment	19	200	37,5	39,5	4
Anoxia pretreatment	19	200	10,5	10,5	1,5

TABLE 3 A. *The effect of 5×10^{-3} M hydrogen peroxide on the frequency of aberrations produced by EOC (10^{-2} M, 1¾ hour treatment, 20° C) in the absence of oxygen.*

EOC, normal aeration ..	5½	200	64,5	14,5	94,5
» no oxygen, no hydrogen peroxide	5½	100	9	4	4
» no oxygen, containing hydrogen peroxide	5½	100	44	13,5	74
Hydrogen peroxide, no oxygen, no EOC	5½	100	0	0	0

TABLE 4 A. *The effect of 5×10^{-4} M sodium azide on the action of normally aerated 10^{-2} M EOC at 10° C.*

EOC, 4 hr, no azide	19	100	64	79	22
» 4 », with azide ..	19	100	1	1	0
» 2 », no azide	22	100	39	41	5
» 2 », with azide pretreatment. See text	22	100	7	5	1
Azide treatment only ...	28	100	4	2	2

TABLE 5 A. *The effect of a pretreatment with 10^{-4} M 2, 4-dinitrophenol on the action of normally aerated 10^{-2} M EOC at 10° C.*

EOC 2 hr, no dinitrophenol	22	200	35,5	39,5	1
EOC 2 hr, with dinitrophenol pretreatment ..	22	170	1,2	1,2	0

See text.

TABLE 1 B. *The effect of anoxia on the reduction in growth of Vicia faba roots after 4 hour treatments with 7×10^{-3} M EOC at 10° C.*

Treatment conditions	Growth made in the 10 days after treatment		Equivalent X-ray dose. See text
	Treated roots	Control roots	
Normal aeration	13,37	23,82	131 r
No oxygen	23,33	23,82	zero

TABLE 2 B. *The effect on growth of a 2 hour period of anoxia immediately before a 2 hour treatment with normally aerated 7×10^{-3} M EOC at 10° C.*

No pretreatment	14,98	20,44	90 r
Repeat	14,41	20,44	96
»	16,14	20,44	80
2 hour anoxia pretreatment	19,45	20,44	45
Repeat	16,23	20,44	79
»	18,26	20,44	59

TABLE 3 B. *The effect on growth of 2 hours in oxygen-free 7×10^{-3} M EOC at 19° C, which contained 5×10^{-3} M hydrogen peroxide.*

No hydrogen peroxide	17,85	19,30	53 r
With hydrogen peroxide	12,18	19,63	113

TABLE 4 B. *The effect on growth of 3 hours in normally aerated 7×10^{-3} M EOC at 10° C when the treatment was modified by 5×10^{-4} M sodium azide.*

EOC only	13,33	24,16	128 r
Azide only, 3 hours	20,90	24,16	41
EOC plus azide in the same solution	21,46	24,16	60
Repeat	23,86	24,16	30
EOC treatment followed 4 hours later by the azide treatment. See text	10,74	24,16	158
Azide treatment followed 4 hours later by the EOC treatment. See text	21,38	24,16	61

TABLE 5 B. *The effect on growth of 2 hours in normally aerated 7×10^{-3} M EOC at 10° C when the treatment was modified by 10^{-4} M dinitrophenol (DNP).*

EOC only	17,03	27,04	111 r
DNP only, 2 hours	25,71	27,04	44

Treatment conditions	Growth made in the 10 days after treatment		Equivalent X-ray dose. See text
	Treated roots	Control roots	
EOC+DNP in the same solution	16,98	27,04	111
Repeat	17,77	27,04	105
EOC treatment followed $2^1/_2$ hours later by the DNP treatment ..... See text	16,72	27,04	113
DNP treatment followed $2^1/_2$ hours later by the EOC treatment ..... See text	23,71	27,04	62

where the yield of isolocus breaks is plotted against oxygen concentration.

To compare with these results, roots have been treated similarly for 3 hours, and their growth in the next 10 days has been measured. Oxygen concentrations of 100, 21 (air), 15, 7,5, and 0 (nitrogen) per cent were used in the bubbling process. The results are shown in Fig. 1 B. The growth reductions obtained are given as the equivalent X-ray doses. Unfortunately, it was necessary to terminate these experiments before an oxygen concentration had been reached which would give a point on the ascending part of the curve, which is therefore dotted in this region to indicate its hypothetical character. Nevertheless, at the five oxygen concentrations used, there is a complete correspondence between the frequencies of aberrations and the relative values of the equivalent doses based on the growth measurements.

According to Table 2 A a 2 hour period of anoxia in tap water before a 2 hour normally aerated EOC treatment causes reduction in aberrations to about one quarter. The equivalent doses from the growth measurements, 2 B, have a mean of 88,5 r, with standard deviation 4,7 r, when no period of anoxia preceded the EOC treatment. The period of anoxia reduced this to 61,0 r, standard deviation 12,2 r. When the »*t*» test is applied it is calculated that the odds are 10 to 1 against the difference being the result of chance.

Tables 3 A and 3 B show that both in regard to the production of aberrations and the reduction in growth an anaerobic EOC treatment in the presence of 5×10^{-3} M hydrogen peroxide produces the same effect as an aerobic treatment with EOC alone.

A concentration of 5×10^{-4} M sodium azide in a normal aerated EOC solution effectively prevents the production of aberrations (Table 4 A). Furthermore, when the roots were immersed for 2 hours in a solution of azide alone 2 hours before the EOC treatment, the intervening period

being spent in water at 17° C, the protection was very nearly as effective. Table 4 B sets out the results of parallel experiments on the growth. The azide was combined with the EOC in a 3-hour treatment, or preceded the EOC treatment by 4 hours or followed it 4 hours later. The intervening periods were spent in tap water at 19° C in the culture tank. The azide almost prevents the reduction in growth when it is present with the EOC, or when the azide treatment is 4 hours earlier, but if it is 4 hours later the growth reduction is a little more than that caused by the EOC treatment alone.

There was only a slight decrease in the production of aberrations when the EOC solution also contained 10^{-4} M, 2,4-dinitrophenol (DNP). However, when roots were immersed in a solution of DNP only for at least 30 minutes before the EOC treatment, there was a marked reduction. Table 5 A shows the effect of a 2 hours immersion in DNP immediately before a 2-hour EOC treatment. A post treatment with DNP did not influence the frequency of aberrations produced by EOC.

Table 5 B shows that treatment with a combined solution of EOC and DNP for 2 hours had no effect on the growth reduction, nor was there any difference when a 2 hour DNP treatment followed a 2 hour EOC treatment with an interval of $2^1/_2$ hours. However, when the DNP treatment preceded the EOC treatment by $2^1/_2$ hours, the reduction in growth caused by the EOC was almost prevented.

In all these cases there is a very close correspondence between the frequencies of chromosome aberrations observed and the reduction in growth of roots which had experienced a similar treatment.

III. QUANTITATIVE COMPARISON OF THE REDUCTION IN GROWTH PRODUCED BY X-RAYS AND EOC AND THE FREQUENCIES OF ABERRATIONS PRODUCED BY THE SAME AGENTS

The previous section shows that there is a qualitative agreement between the production of aberrations by EOC and the reduction in growth of the roots. A similar qualitative agreement had been found when X-rays were used. This prompted the question: »Suppose we reduce the growth of roots to the same degree by X-rays on the one hand, and by EOC on the other, will 2 groups of roots similarly treated carry the same number of chromosome aberrations?» This was investigated as follows.

We already had available data on the production of chromosome aberrations by X-rays.

THODAY (1951) tabulated the percentages of abnormal anaphases obtained at various times after treatments of *Vicia faba* root tip cells with 3 doses of X-rays (0,85 mm Cu H. V. L.). The treatments were given to roots in normally aerated water at 19° C, and in a period of 4 minutes. His values for 210 r and 105 r are graphed in Fig. 2. He also tabulated the numbers of fragment nuclei observed per 100 cells. They are plotted in our Fig. 3.

The work of GRAY and SCHOLES (*loc. cit.*) and unpublished work by READ has shown that the difference in growth response after X-ray doses of this order of magnitude given in 4 minutes and in 10 minutes is too small to be detected. READ (1952) has established a curve which relates the growth made in the 10 days after an X-ray dose given in 10 minutes and the magnitude of the dose. By the use of this it has been determined that a 4-hour treatment in normally aerated EOC solution at 10° C is equivalent to $135 \pm 9{,}3$ r. This value is bracketed by the doses used by THODAY so this treatment was adopted for the comparison.

Twenty-seven roots were first treated of which 9 were reserved for growth during the next 10 days to check the X-ray equivalence. Twenty-four were then treated in a second batch and a second set of 9 were reserved for growth. The others were fixed in a 3 : 1 alcoholic-acetic acid fixative after the same time intervals as those used by THODAY. In these intervals the roots grew in the culture tank under the same conditions as those in the early experiments. After the last fixation, the sets of tips were each sealed in the fixative in a glass ampoule and sent by air mail from READ at Dunedin, New Zealand, to KIHLMAN at Baltimore, U. S. A. One slide was prepared from each root tip as a Feulgen squash and the percentages of abnormal anaphases and of micronuclei (fragment nuclei according to THODAY) were scored. For the anaphase data cells from all 3 slides for each fixation time were grouped together, and when possible 200 cells were analyzed. To determine the frequencies of micronuclei, 2000 cells were counted in 9 areas of 2 slides (1000 cells per slide) at each fixation time. The percentages of abnormal anaphases are plotted in Fig. 2 and of micronuclei on Fig. 3.

The stippled area in the EOC graph on Fig. 2 shows the number of anaphases which were abnormal as a result of »sticky bridges», and they should be deducted before a comparison is made with the X-ray curves of the same Fig. The graph for the EOC treatment then lies between those for 210 and 105 r, being closer to the curve for 105 r.

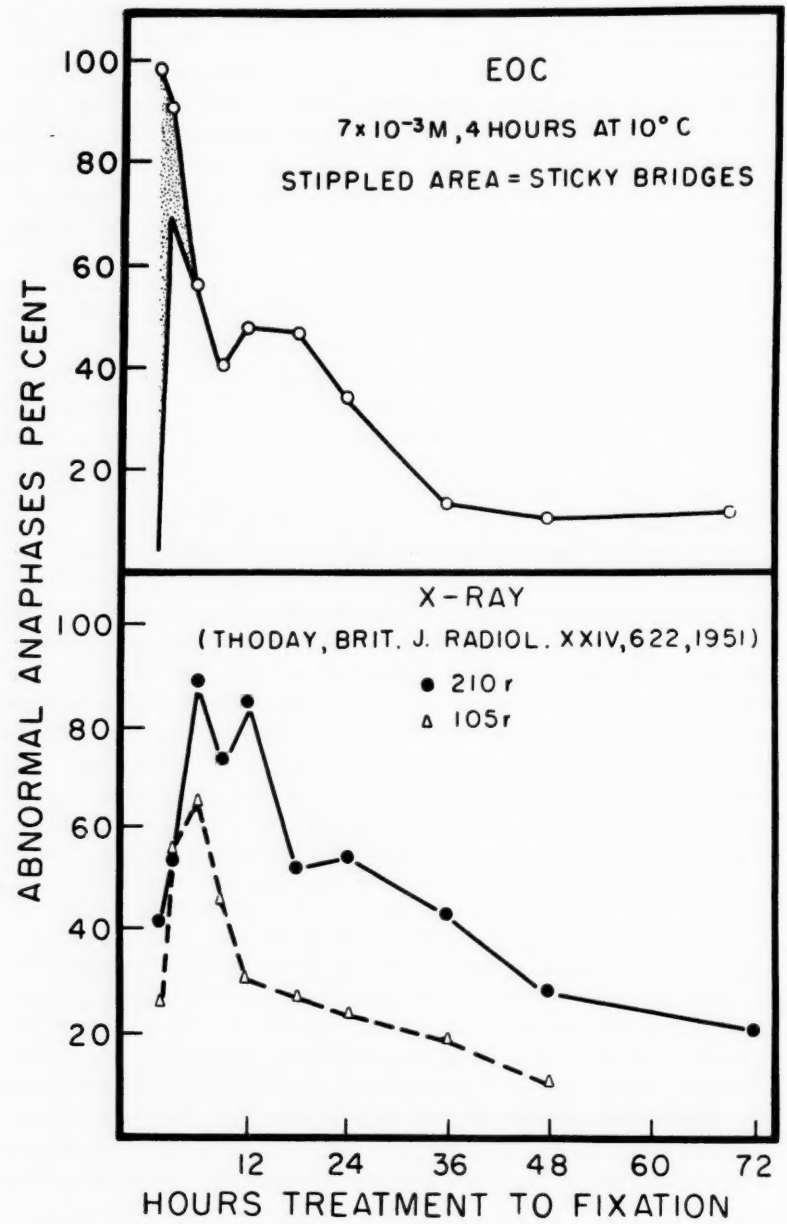

Fig. 2. The percentages of abnormal anaphases obtained various times after treatment with EOC and X-rays.

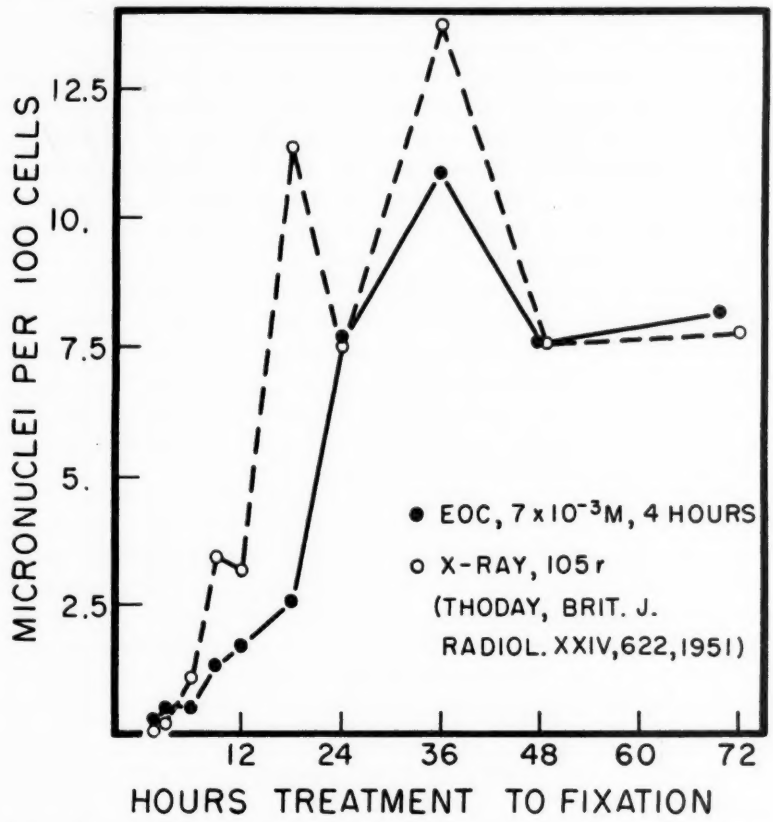

Fig. 3. The frequencies of micronuclei observed various times after treatment with EOC and X-rays.

Fig. 3 shows the frequencies of micronuclei produced by the EOC treatment and by 105 r of X-rays in THODAY's experiment. During the first 18 hours of the recovery period the percentages of micronuclei produced by the EOC treatment are considerably lower than those poduced by 105 r of X-rays. From 24 hours onwards the frequencies are about the same in the two cases. The frequency of micronuclei observed at a certain time after treatment indicates the number of cells with broken chromosomes which have divided during the period between treatment and fixation. Therefore, it is dependent not only on the percentage of

abnormal divisions but also on the frequency of mitoses. Since the percentages of abnormal anaphases produced by the EOC treatment actually are higher than that produced by 105 r, the comparatively low frequency of micronuclei during the first hours of the recovery period is probably due to a lower mitotic activity after the EOC treatment. KIHLMAN (unpublished data) has shown that EOC actually induces a stronger inhibition of mitosis during the first 12 hours of the recovery period than an X-ray treatment which produces the same frequency of abnormal anaphases as the EOC treatment.

Thus, the cytological damage produced by the EOC treatment appears to be of the same magnitude (data based on micronuclei) or slightly higher (data based on abnormal anaphases) than that obtained by THODAY after treatment with 105 r of X-rays. READ's growth measurements on the 2 sets of 9 roots which had been treated with the roots for the cytological study corresponded to X-ray doses of 129 r and 119 r in satisfactory agreement with the anticipated 135 r.

Having regard to the number of steps which intervene in the comparison of THODAY's observations made in the late 1940's and those recorded in this paper, the measure of agreement is striking, and, we believe, entitles us to claim that there is a close quantitative agreement between the proportion of cells which carry chromosome aberrations and the reduction in growth of roots similarly treated, no matter whether the agent responsible is radiation or EOC.

IV. THE VARIATIONS IN GROWTH RATE OF ROOTS AFTER AN EOC TREATMENT

READ (1954) has shown that in the case of X-rays the growth rate of broad bean roots begins to drop about 24 hours after the treatment. If roots are treated with nitrogen mustard the drop is delayed by a further period of about 24 hours. READ suggested that this was a consequence of the difference in the sensitive period of the chromosomes to the two injurious agents. REVELL (1953) had earlier shown that aberrations were produced in the chromosomes more readily just before mitosis if X-rays were used, but in contrast to this, in the early resting stage when they were caused by a diepoxide treatment, which in all respects is very similar to a nitrogen mustard treatment. The general accuracy of the correlation of growth rate and chromosome sensitivity has been tested by obtaining a growth rate curve after an anaerobic X-ray irradiation, after an alpha ray irradiation by the method of GRAY and READ (1942),

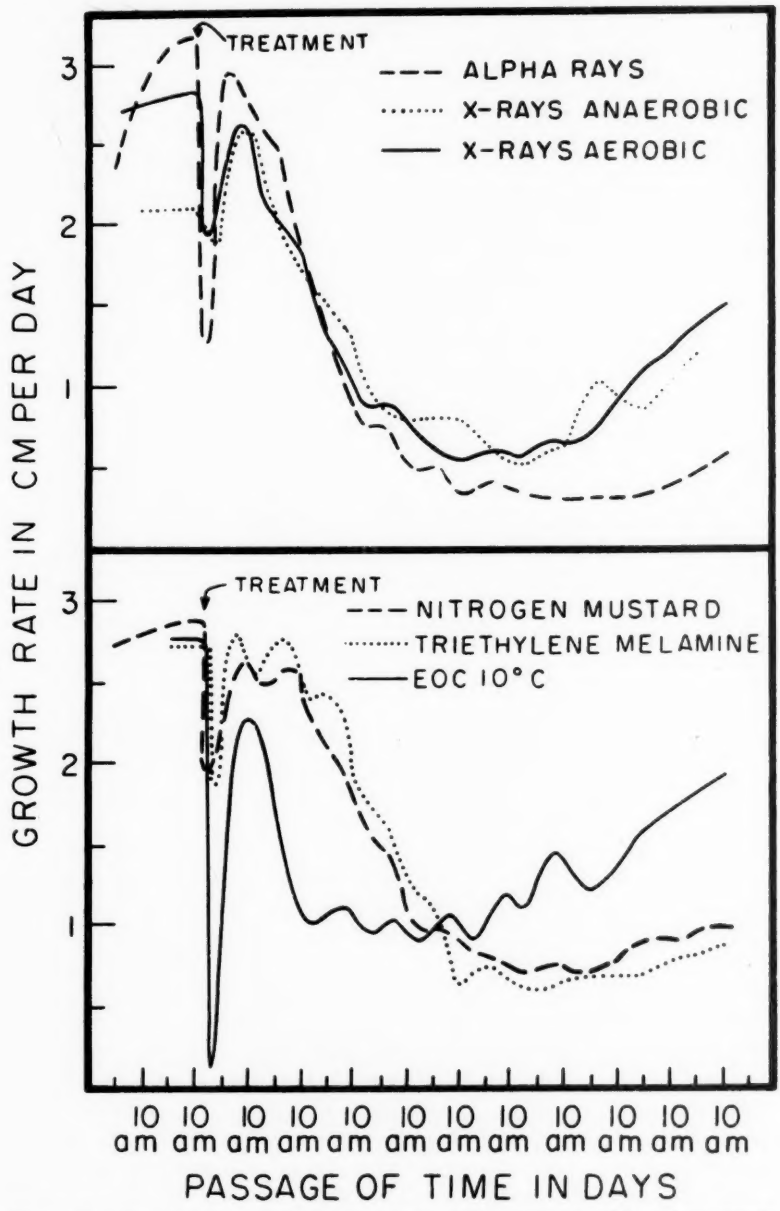

Fig. 4. Growth rate curves of *Vicia faba* roots after treatments with various agents.

and after a treatment with triethylene melamine. The results are shown in Fig. 4. The three curves for the ionizing radiations fall almost on top of each other, but the curve for triethylene melamine (which is believed to be in the same class of mutagenic agents as diepoxide and nitrogen mustard) is very close to that for nitrogen mustard. The sixth curve was drawn from measurements on roots which had been immersed in a normally aerated 7×10^{-5} M EOC solution for 3 hours. The curve for EOC is definitely of the X-ray type with two minor differences. In all six cases there is a sudden drop in growth rate in the few hours immediately after treatment, followed 24 hours later by a recovery approximately to the pre-treatment value. After the 3 hour EOC treatment the recovery was somewhat less and was still less after a 4 hour treatment. The second difference is that the ultimate recovery from the major growth reduction occurs sooner after EOC treatments. The minimum is reached during the third and fourth day and an upward trend starts at the end of the fifth day. In the case of the ionizing radiations the minimum growth reduction occurs during the sixth day after irradiation. The finding that recovery occurs sooner in the case of EOC is paralleled by the cytological observation that the frequency of abnormal cell divisions decreases more rapidly after EOC treatments than after X-ray treatments.

KIHLMAN (1955 a) showed that the chromosomes of *Allium cepa* were most sensitive to EOC when the cell was just a stage prior to the onset of mitosis. The same interphase sensitivity to chromosome breakage by EOC was found in a subsequent study with *Vicia faba* as experimental material (KIHLMAN, 1955 b). It is evident, at least in so far as interphase sensitivity is concerned, that the effect of EOC is more similar to that of X-rays than it is to the radiomimetic effect of nitrogen mustard. It appears, therefore, that the correlation between the occurrence of the reduction in growth rate after a treatment and the interphase sensitivity to chromosome breakage, is also typical of EOC (the sixth case).

As a whole the six curves of Fig. 4 afford strong support for the belief that it is not until cells reach the dividing stage that the injury caused by X-rays and radiomimetic chemicals (including EOC) makes itself evident. Furthermore, the onset of this manifestation of injury is correlated with the interphase sensitivity to chromosome breakage, being soon after treatment when the most sensitive period is just before mitosis, and delayed by an appreciable fraction of the intermitotic period when the most sensitive period is in early interphase.

V. COMPARISON OF THE INFLUENCE OF THE DURATION OF TREATMENT BY X-RAYS AND BY EOC

It is convenient to express the effect of an EOC treatment in terms of the X-ray dose which would have produced the same reduction in growth when given to roots in normally aerated water at 19° C in a period of 10 minutes (READ, 1952). All the values so obtained for treatments with 7×10^{-3} M normally aerated EOC at 10° C are collected together in Fig. 5. The means are plotted with open circles and the

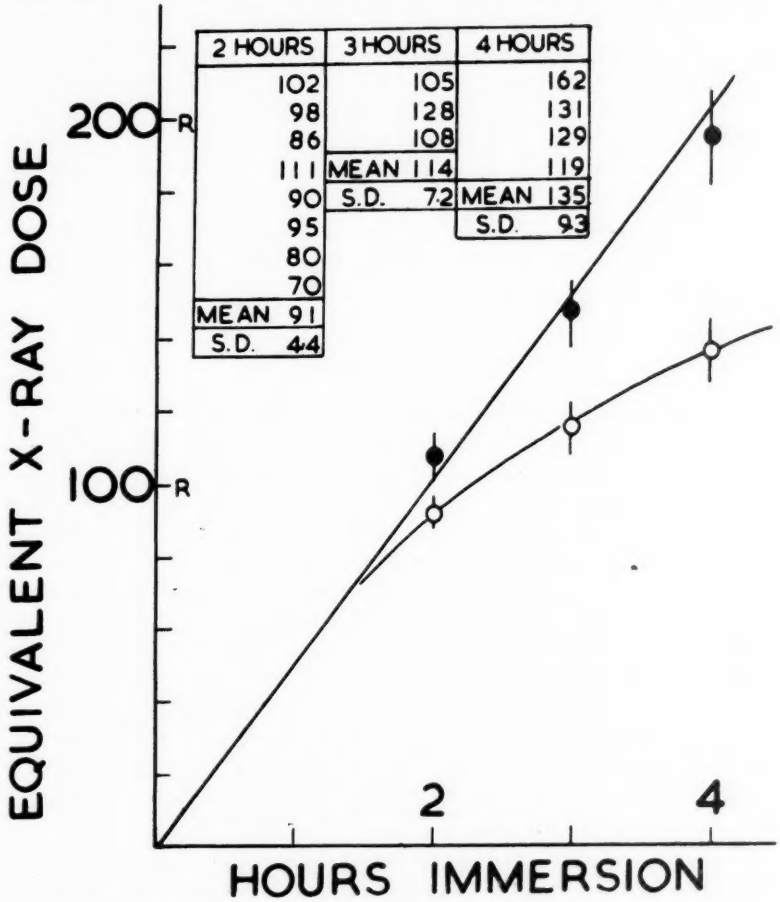

Fig. 5. The influence of the duration of an EOC treatment on its effect.

resulting graph is far from linear with duration of treatment. The effectiveness of the EOC falls below proportionally in a way rather similar to that of X-rays which was described by GRAY and SCHOLES (*loc. cit.*). Their Fig. 27 contains a curve relating to X-ray exposures of 8 minutes which is sufficiently close to the period of 10 minutes adopted by READ. With the aid of this Fig. 27 we can calculate what values X-ray doses must have when given in 2, 3, and 4 hours so that the effective doses given in 8 minutes have the same values as those graphed with open circles in our Fig. 5. These calculated doses are plotted with full circles and lie in a straight line passing through the origin. That is, if we assume for the moment that the »dose» of a constant concentration of EOC can be measured by the product of concentration and duration of treatment, the effectiveness of the »dose» falls below proportionality in the same way as the effectiveness of an X-ray dose given at a constant intensity. No doubt this diminution in effectiveness is the result of biological factors and not an attribute of the X-rays or EOC. Furthermore, the degree of equality in the fall-off when X-rays and EOC treatments are given over extended periods suggests that the same biological factors are involved in the two cases.

VI. DISCUSSION

The experiments described above clearly indicate that there is a close correlation between the degree of chromosome damage and growth inhibition produced by EOC. When the environment of the roots during an EOC treatment is such that few, if any, chromosome aberrations are produced, there is no detectable reduction in the subsequent growth of the roots. If a different environment leads to a large number of aberrations there is a marked reduction, and if an intermediate number is produced growth reduction is also intermediate.

It must be pointed out, however, that this is true only when the treatments are performed at low temperatures. At 10° C, the major cytological effect of EOC seems to be the production of chromosome aberrations. When the treatments are performed at higher temperatures (20—29° C), the radiomimetic effect of EOC is accompanied, and sometimes obscured, by other effects, which, for lack of a better term we propose to call »unspecific toxic effects».

These toxic effects are of at least two different kinds. One results in the death of meristematic cells during or shortly after the treatment. It is increased both by anoxia and by high temperatures. It is probably

due to a coagulation of nuclear and cytoplasmic proteins by EOC, and every factor which decreases the stability of the colloidal state of the proteins increases this effect of EOC. This immediate toxic effect usually affects all cells in the meristem and, thus, results in the death of the whole root. Sometimes only a part of the cells are effected, however, and in these cases the toxic effect, no doubt, manifests itself macroscopically as a reduction in growth of the root.

The second toxic effect has been observed within two days after the EOC treatment. It affects the elongating zone which becomes black and slimy. This toxic effect, which is decreased by anoxia, also results in the death of the root.

To summarize, under treatment conditions which favour the toxic effects of EOC (*e. g.* high temperature), death of roots and inhibition of growth may be due to factors other than chromosome damage. But when these complications are eliminated by performing the treatments at low temperature, the same close correspondence exists between chromosome damage and reduction in growth after EOC treatments as that which had been observed previously after treatments with ionizing radiations (GRAY and SCHOLES, *loc. cit.*).

EOC, like ionizing radiations, produces a temporary inhibition of cell division. The mitotic inhibition is greater than that produced by an X-ray treatment which results in the same amount of chromosome damage. It is not influenced by changes in environment in the same way as the production of aberrations. Like the toxic effects, but in contrast to the effect on the chromosomes, the mitotic inhibition is increased by an increase in temperature. Furthermore, the inhibition of mitosis produced by EOC has been found to be relatively unaffected by oxygen tension.

Thus, in the case of EOC, there seems to be little correspondence between the effect of environmental changes on the degree of chromosome damage on the one hand and the degree of mitotic inhibition on the other. It has also been observed that EOC and X-ray treatments which result in the same amount of chromosome damage, produce the same inhibition of growth, but entirely different effects on mitosis. This is interesting because somewhat similar observations have been made in the case of ionizing radiations. GRAY and READ (1950) pointed out that whereas the relative efficiencies of gamma rays, neutrons, and alpha rays in causing cessation of growth of the roots of *Vicia faba* during a 10-day period following irradiation were in the ratios of 1 : 9 : 9, their relative efficiencies in inhibiting mitosis 3 hours after treatment were

quite different, *viz.*, 1 : 2 : 0,5. No decisive conclusion could be drawn from these data because the sequence of changes in the mitotic index after an alpha ray dose had not been determined. However, taken in conjunction with these new observations, it seems very probable that mitotic inhibition, as such, is not a factor of significance in producing the permanent cessation of growth or the reduction in growth measured over a period as long as 10 days after treatment.

CANTI and SPEAR (1929) have shown that after small doses of gamma radiation the inhibition of mitosis of chick fibroblasts *in vitro* is subsequently compensated by a corresponding excess of dividing cells. At higher dose levels the degree of compensation is smaller. TANSLEY, SPEAR and GLÜCKSMANN (1937) found that degenerate cells appear in rat retina after irradiation at a time which more or less coincides with the period of high mitotic activity which marks the recovery from the radiation induced inhibition of mitosis. These cells break down before metaphase and therefore have not been scored by the methods of THODAY and KIHLMAN which are restricted to metaphase and anaphase cells. However, effects similar to those described by SPEAR and his coworkers have been observed also after treatment of root tip cells of *Vicia faba* by X-rays and EOC (KIHLMAN, unpubl.). We conclude that in the case of *Vicia faba* compensation of the initial mitotic inhibition is complete within the 10 day period after treatment, except so far as cells degenerate from injuries unrelated to mitotic inhibition. The coincidence in time between the compensatory increase in mitotic activity and the appearance of degenerate cells would then be due to the fact that the injury does not become evident until the affected cells attempt to divide. Mitotic inhibition would not alone constitute a permanent injury. This, at least, can be said with certainty about the injuries which eventually lead to a reduction in growth or to a permanent cessation of growth of the roots after irradiation and after treatments with EOC at low temperature: *Whatever factors increase or diminish the numbers of cells carrying chromosome aberrations also increase or diminish the injuries on which the macroscopic effect depends, and to the same degree.*

While we lack evidence to the contrary and have this abundant evidence of an exact correspondence between the amount of chromosome damage and the overall macroscopic damage, it seems reasonable to conclude that the chromosome damage is, in fact, the cause of the latter. On this view, the cells observed to degenerate before metaphase must be in proportion to those whose aberrations can be observed at metaphase and anaphase and presumably are those which have suffered

such severe chromosome damage that progress in mitosis as far as metaphase is not possible. It is not believed that the death or reduced growth of the roots is an immediate consequence of cell death from chromosome damage, as no doubt other steps intervene. GRAY and SCHOLES (*loc. cit.*) concluded that the reduction in growth was mainly due to a slowing up of the progress of cells from one division to the next. It seems likely to us that this slowing up is a secondary effect which results from the death of a relatively small proportion of cells, in such a way that there is a rough proportionality between the gross overall effect and the number of cells killed. The release of breakdown products from the dead cells, which permeate the main body of normal cells, might bring this about.

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J. READ.

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B. A. KIHLMAN.

SUMMARY

The work of GRAY and his co-workers has shown that chromosome structural damage constitutes a major factor leading to growth inhibition of *Vicia faba* root tips by ionizing radiations.

The present study was performed in order to find out if a similar correlation between chromosome damage and growth inhibition exists when root tips of *Vicia faba* are treated with the radiomimetic chemical 8-ethoxycaffeine (EOC).

In the first section, a comparison is made between the modifying influence of oxygen tension, hydrogen peroxide, sodium azide, and dinitrophenol on the production of chromosome aberrations on the one hand and the reduction in growth, on the other. It was found that these

factors modified the frequency of chromosome aberrations produced by a given EOC treatment in the same direction and to the same degree as they affected the reduction in growth.

In the second section of the present paper, the reduction in growth produced by treatments with X-ray and EOC is compared with the frequencies of aberrations produced by the same treatments. The results indicated a close quantitative agreement between the proportion of cells carrying chromosome aberrations and the reduction in growth of roots similarly treated, no matter whether the agent responsible is X-rays or EOC.

In the third section the variation in growth rate after EOC treatments is compared with that after treatments with α-rays, X-rays, nitrogen mustard, and triethylene melamine. It was found that the onset of the growth inhibition is correlated with the interphase sensitivity to chromosome breakage, being soon after treatment when the most sensitive period is just before mitosis, and delayed by an appreciable fraction of the intermitotic period when the chromosomes are most sensitive to breakage at early interphase. These facts are in agreement with the hypothesis that it is not until cells reach the dividing stage that the injury caused by X-rays and radiomimetic chemicals makes itself evident as a reduction in growth.

The experiments described in the fourth section show that when EOC or X-ray treatments are given over extended periods, the effectiveness of the »dose» decreases proportianally in the same way in the two cases.

The results are discussed in a final section. It is concluded that when *Vicia faba* root tips are treated with X-rays or with EOC at 10° C, chromosome structural damage is the major factor responsible for the reduction in growth of the roots.

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DIFFERENTIAL PERIODICITY AMONG CLONES OF CACAO

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AS a result of the pioneer work carried out at the Imperial College of Tropical Agriculture in Trinidad (*cf.* CHEESMAN, 1946), a change from seed reproduction to vegetative propagation is characteristic of present-day cacao cultivation in South and Central America. As the seedling fields of cacao are generally very uneven, comprising many individuals with poor fruit-setting, clonal propagation of selected plus individuals may obviously lead to very marked increases in yield. At the same time constant cacao varieties are being obtained, the properties of which may be accurately defined.

In Ecuador cacao production was flourishing until about 1920 when two dangerous diseases, *Monilia* and the witches' broom disease, *Marasmius perniciosus*, began to cause a disastrous decline in the cacao yields (*cf.* ERNEHOLM, 1948). This decline had also severely affected cacao production at the hacienda Clementina, situated at the foot of the Andes, near the town of Babahoyo. This hacienda is owned by a Swedish company (A.B. Clementina, Stockholm), and about ten years ago the directors decided to try to raise cacao production there with the help of modern agricultural methods. The present writer suggested a change from seed reproduction to clonal propagation in order to get clones with high yield, disease resistance and good quality. With this end in view, work was started in 1946 with a selection of clones from local populations occurring at the hacienda. Shortly afterwards a fairly large clonal material was generously put at our disposal by the agricultural authorities of Trinidad.

After a period of propagation two different field trials were planted in December 1951 with some supplementary planting in the beginning of 1952. One of these trials is a disease-resistance trial located in a heavily infected area, the other trial being designed to give information about the specific yield of the various clones. This trial comprises four blocks, each block containing 14 different clones and, in addition, 2 standard clones. These latter clones are present in all four blocks in

TABLE 1. *Percentages of the total cacao yield (gr. dry cacao) produced during the four quarters of the year. Data from ICS 1 and the 10 most high-yielding cacao clones.*

Clone	Quarter			
	1	2	3	4
ICS 1, a	3,0	15,8	6,5	74,8
» , b	1,0	13,0	18,8	67,4
» , c	1,1	14,4	28,8	55,6
» , d	0,0	6,8	28,7	64,6
ICS 1, average	1,3	12,6	20,7	65,6
CG 12, a	16,3	52,3	6,4	24,8
» , b	12,5	46,2	21,7	19,5
» , c	9,5	58,2	21,4	10,9
» , d	10,5	45,9	23,3	20,2
CG 12, average	12,3	50,7	18,2	18,9
S 62	22,1	40,4	19,4	18,1
CG 17	7,0	33,3	29,0	30,8
SCA 12	20,1	50,2	22,3	7,5
ICS 6	1,1	13,3	16,3	69,4
» 8	3,2	23,6	27,3	46,0
» 28	9,8	59,8	5,0	25,2
» 46	14,5	42,4	29,3	13,8
» 78	15,4	52,1	5,4	27,0
» 95	1,6	28,9	38,2	31,4

order to enable indirect comparisons between clones of different blocks. In each block every clone is represented by 32 trees, distributed over 32 different plots. In each plot, containing 16 plants from 16 clones, the arrangement of the trees is at random.

In clone plants of cacao, fruit-setting may commence as early as the third year after planting, and in the fourth year about 75 per cent of the trees in our yield were in production. The data obtained enabled us to make preliminary ranking lists with regard to yield and disease resistance. During this work striking clone differences in the periodicity of fruit production were observed. These observations are limited to the ten most high-yielding clones and to the standard clone ICS 1. From these 11 clones monthly records were taken during the year 1955, or, more precisely, during the interval December 15, 1954 to December 15, 1955. For each of the eleven clones the monthly records comprised

number of healthy and diseased pods harvested and grams of dry cacao obtained. It was found to be convenient to concentrate the monthly values to quarter year values which are given in Tables 1 to 3.

Of the clones represented in the table those with the denomination ICS (»Imperial College Selection») are from Trinidad. CG 12 and CG 17 are local selections from Clementina, S 62 is a clone from another part of Ecuador and SCA 12 is one of the witches broom-resistant clones selected by POUND in the upper Amazon region (*cf.* POUND, 1938, 1943).

The values in Table 1 represent the percentages of the total cacao yield (gr. dry cacao) produced during the four quarters of the year. Being a standard clone, ICS 1 is represented in all four blocks of the trial, a, b, c, and d, and in each block by 32 trees. The same is true of CG 12, a local selection from Clementina, which (in contrast to ICS 1) also belongs to the 10 most high-yielding clones. Thus, the average values of ICS 1 and CG 12 are based on data from $4 \times 32 = 128$ trees and are consequently more reliable than the values of the other 9 clones, which are based on 32 trees, growing in one block only.

A comparison between the average series of ICS 1 and CG 12 reveals a striking difference in periodicity. Whereas in the former clone not less than 65,6 per cent of the yield was produced in the fourth quarter, the corresponding value of CG 12 is only 18,9. This clone instead had its maximal production (50,7) in the second quarter. This difference is not only evident from the average values but is equally clear in the values from each separate block.

Among the other nine clones there were also obvious differences. Two of the ICS clones, 6 and 8, were similar to ICS 1, having a clear peak of production in the fourth quarter, whereas ICS 28, 46 and 78 had their maxima in the second quarter just as CG 12. ICS 95 seems to have a more even production without any clear maximum.

The yield of dry cacao is predominantly obtained from healthy pods. Especially during the rainy season, which at Clementina occurs in the months of January to May, the percentage of diseased pods may be considerable. Under such circumstances more accurate information on the type of periodicity may be obtained from data on the total number of fruits produced rather than from data on the weight of dry cacao.

Table 2 is based on the total number of pods harvested, both healthy as well as diseased. The general trend is the same as in Table 1 but one difference is clearly noticeable. In quarters 1 and 2, roughly representing the wet half of the year, the values in Table 2 are as a rule higher than the corresponding values of Table 1. Vice versa, the values of quarters 3

TABLE 2. *Percentages of the total number of pods (healthy and diseased) produced during the four quarters of the year. Data from ICS 1 and the 10 most high-yielding cacao clones.*

Clone	Quarter			
	1	2	3	4
ICS 1, a	6,1	14,7	7,8	71,5
» , b	3,2	14,7	19,2	62,9
» , c	1,6	20,2	29,0	49,2
» , d	2,7	13,5	26,0	57,8
ICS 1, average	3,4	15,8	20,2	60,5
CG 12, a	23,1	50,7	5,6	20,6
» , b	12,3	48,4	21,9	17,4
» , c	11,0	59,8	21,5	7,7
» , d	10,6	44,9	23,6	20,8
GG 12, average	13,4	51,5	19,4	15,8
S 62	19,1	45,3	19,6	16,0
CG 17	7,3	36,7	26,2	29,8
SCA 12	20,8	53,2	19,3	6,6
ICS 6	4,3	13,2	16,2	66,4
» 8	6,1	25,5	27,6	40,8
» 28	20,6	52,9	5,5	20,9
» 46	16,3	44,4	27,8	11,5
» 78	32,6	45,0	4,8	17,6
» 95	3,9	29,6	37,2	29,4

and 4, representing the dry season, are lower in Table 2 than in Table 1. Thus, with regard to the average values of ICS 1, 3,4+15,8=19,2 per cent of the cacao pods of the year were harvested during the first two quarters, but these pods produced only 1,3+12,6=13,9 per cent of the annual yield of dry cacao. To take a more extreme example, ICS 78 produced 32,6+45,0=77,6 per cent of the pods during January to June but only 67,5 per cent of the total cacao yield.

This discrepancy, which is more or less pronounced in all the clones, is simply due to the fact that a higher proportion of the pods are destroyed by various diseases during the rainy season than during the dry season. Thus, during the wet period a relatively larger number of pods are needed to produce a certain amount of dry cacao than during the dry period when the diseases are at a minimum.

Direct data on the percentages of diseased pods during the four

TABLE 3. *Percentages of diseased pods during the four quarters of the year. Data from ICS 1 and the 10 most high-yielding cacao clones.*

Clone	Quarter				The whole year
	1	2	3	4	
ICS 1, a	(59,1)	13,2	(21,4)	0,0	7,2
» , b	(80,0)	29,4	4,5	0,0	10,8
» , c	(66,7)	39,5	7,3	1,6	12,0
» , d	(100,0)	(57,5)	5,2	0,0	11,8
ICS 1, average	72,5	33,8	10,6	0,8	10,5
CG 12, a	33,3	7,3	(5,1)	2,8	12,3
» , b	27,4	12,3	10,7	0,4	11,7
» , c	37,0	15,2	3,4	0,0	13,1
» , d	10,0	12,3	8,0	0,0	8,5
CG 12, average	28,6	11,9	7,5	0,8	11,4
S 62	9,8	17,5	11,0	1,3	12,1
CG 17	21,1	7,5	10,2	2,2	7,3
SCA 12	15,5	6,4	4,2	1,5	7,6
ICS 6	(81,0)	13,8	16,3	0,0	7,9
» 8	(66,0)	27,8	14,5	1,9	15,9
» 28	64,8	7,1	(26,3)	2,8	19,2
» 46	29,4	15,1	9,5	(4,2)	14,6
» 78	64,1	21,5	21,1	2,9	32,1
» 95	(67,7)	23,8	5,8	4,7	13,2
All ICS clones	63,6	20,4	10,4	2,5	16,2
The other clones	18,8	10,8	8,2	1,5	9,6

quarters of the year are given in Table 3. Values based on less than 50 pods are put in brackets. The differences between the clones are very striking. Thus, in ICS 1 not less than 72,5 per cent of the pods were diseased during the first quarter, the values of the other three quarters being 33,8; 10,6 and 0,8 per cent, respectively. For the whole year the average percentage of diseased pods is not higher than 10,5, as most of the pods were produced during the dry season.

In CG 12 there is a similar trend, the values of the four quarters being 28,6; 11,9; 7,5, and 0,8 per cent, respectively, with a total year average of 11,4. It is evident, however, that during the rainy season CG 12 shows a much better resistance than ICS 1, which is then very heavily attacked. On an average the other ICS clones behaved in a similar way, showing more or less high percentages of diseased fruits during the first two

quarters. All these clones had higher values for the rainy season than any of the other four clones. This difference is again obvious in the average values at the bottom of Table 3. During the first two quarters the seven ICS clones had 63,6 and 20,4 per cent of diseased pods, respectively, the corresponding values of the other four clones being 18,8 and 10,8. As three of these latter clones are from Ecuador and the fourth one from an adjoining region, there is some reason to believe that these clones are better adapted to the Clementina climate than the ICS clones. At any rate, the pods of the Ecuadorian clones are much less subject to destruction by disease during the rainy season than the ICS clones, which during this time suffer very heavy losses. In fact, several of these clones, including ICS 1, would probably be impossible to use at Clementina, if their periodicity had not been such that most of their yield is produced during the dry season.

Now, the periodicity figures are certainly preliminary and a longer period of observation is needed before entirely reliable conclusions can be drawn. It can, however, be predicted that future values will tend to be higher for the first quarters and lower ones for the last quarters than those now presented. This will be so because the observed material was quite young and only in its fourth year. Under such circumstances production will of course tend to be higher at the end of the year when the threes were older than in the beginning of the year. However, in spite of this source of error, all the Ecuadorian clones, SCA 12 and three of ICS clones, and their maximum production in the second quarter. Thus, although inherent differences in periodicity are obvious, more data are necessary for their clear definition.

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SUMMARY

In a material of cacao clones, tested at Hacienda Clementina in Ecuador, monthly pod production was recorded for eleven clones, obvious differences in periodicity being observed. Three clones from Ecuador and one from the upper Amazon region had their maximum

production in the second quarter of the year, during or shortly after the rainy season. Though the rainy period favours fungus attacks, the percentage of diseased pods during this time was relatively low in the Ecuadorian and Amazon clones. In seven clones from Trinidad, on the other hand, the percentages of diseased pods were quite high during the rainy season. Therefore, only such Trinidad clones that have their maximum production during the dry season seem to be useful in the type of Ecuadorian climate obtaining at Clementina.

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BRIEF REPORTS

L. EHRENBERG und K. G. ZIMMER: Die Bedeutung der Mikrowellenspektroskopie für die Strahlenbiologie.

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Eines der wenigen allgemein gültigen Ergebnisse der Strahlenbiologie besteht in der Erkenntnis, dass die Frage nach *'dem* Mechanismus *der* biologischen Strahlenwirkung' falsch gestellt ist. Es gibt viele Arten biologischer Strahlenwirkungen, und alles spricht bisher dafür, dass sie nach vielen verschiedenen Mechanismen ablaufen. Daher sind auch alle Versuche gescheitert, in denen bewiesen werden sollte, dass alle biologischen Strahlenwirkungen durch Einwirkung auf Sulfhydrylgruppen, durch in der Zelle gebildetes Wasserstoffsuperoxyd oder durch die bei Bestrahlung in Wasser gebildeten Radikale hervorgebracht werden. Es gibt für und gegen alle diese Ansichten mehr oder weniger zahlreiche und eindeutige Beispiele. Andererseits ist die Lösung der bereits 1920—1925 viel diskutierten Frage (DESSAUER, 1922, HOLTHUSEN, 1924) noch in keinem Falle gelungen, mittels welcher Mechanismen so geringe Beträge absorbierter Energie so grosse biologische Wirkungen hervorbringen. Diese Frage wird neuerdings, und sehr berechtigter Weise, wieder stärker betont (BUTLER, 1956), und es ist interessant zu bemerken, dass sich auch heute noch kein anderer Ausweg gefunden hat, als die Annahme der Beeinflussung von 'Molekülen', die je Zelle nur in einem oder sehr wenigen Exemplaren vorkommen (MCILWAIN, 1946). An anderer Stelle wurde bereits dargelegt (ZIMMER, 1956), dass sich in den letzten Jahren überraschend viel Material dafür ergeben hat, dass den sogenannten 'direkten' Strahlenwirkungen[1] eine erheblich grössere Bedeutung zukommt, als manche unter dem Eindruck der sehr schnellen Entwicklung der Strahlungschemie verdünnter wässriger Lösungen glaubten. Es wurde auch darauf hingewiesen, dass sich einige Ansatzpunkte für eine Konkretisierung des Mechanismus der direkten Strahlenwirkung ergeben haben. Die Beschreibung eines Treffers als Energiezufuhr zu einem 'Molekül' mit anschliessender Umlagerung desselben (TIMOFÉEFF-RESSOVSKY, ZIMMER und DELBRÜCK, 1935, LEA, 1946, TIMOFÉEFF-RESSOVSKY und ZIMMER, 1947) war von vielen Chemikern und Biologen als zu formal und daher unbefriedigend empfunden worden. Ausserdem war die Beeinflussbarkeit zahlreicher biologischer Strahlenreaktionen durch den Saurstoffdruck und durch Zugabe meist

[1] Es erscheint ebenso notwendig wie schwierig, den vielbenutzten Begriff 'direkte' Strahlenwirkung eindeutig zu definieren. Wir glauben nicht, dass der Stand unseres Wissens im Zusammenhang mit der Strahlenbiologie eine Diskussion von Dimensionen unter etwa 100 Å, ausser in Sonderfällen, aussichtsreich und sinnvoll erscheinen lässt. Wir würden daher jede Wirkung vorläufig als direkt bezeichnen, zu der Energieabsorption ausserhalb eines Volumens dieser Dimension oder ausserhalb eines Mantels von etwa 100 Å Dicke um die betrachtete biologische Struktureinheit *nicht* beiträgt; als indirekt jedoch eine Wirkung, zu der Energieabsorption beiträgt, die primär in grösserer Entfernung erfolgt und durch Sekundäreffekte (Energieübertragung, Diffusion etc.) wirksam wird.

schwefelhaltiger 'Schutzstoffe' von vielen als Beweis gegen die Bedeutung 'direkter' Treffer gewertet worden, wobei jedoch gelegentlich ganz richtig betont wurde, dass auf diese Effekte gegründeten Überlegungen mehr die Bedeutung eines Arguments als eines Beweises zukomme (BACQ und ALEXANDER, 1955).

Es erscheint uns nicht lohnend, an dieser Stelle ausführlich die verschiedenen Beobachtungen zu besprechen, die zum Beispiel mit einer alle anderen Wirkungsmechanismen überragenden Bedeutung der durch Strahlen im Wasser gebildeten Stoffe schwer vereinbar sind. Es sei nur an die mit steigendem Wassergehalt geringer werdenden Strahleneffekte bei manchen Organismen und hydrophilen Kolloiden (LAMBERT, 1933, KAPLAN, 1940, EHRENBERG, 1954, CALDECOTT, 1954), an den sorgfältigen Nachweis des Nichtbeteiligtseins von Wasserstoffsuperoxyd an dem durch Röntgenstrahlen induzierten Mutationsprozess (HEARON und KIMBALL, 1955, KIMBALL, HEARON und GAITHER, 1955), sowie an die seit mehr als 10 Jahren bekannten, aber oft nicht berücksichtigten Überlegungen erinnert, dass Zellinhalte keine verdünnten wässrigen Lösungen sind (LEA, 1946, TIMOFÉEFF-RESSOVSKY und ZIMMER, 1947).

Vielmehr glauben wir, dass neue Ergebnisse und Denkmöglichkeiten von Wichtigkeit sind, die es erlauben, die Mechanismen der direkten Strahlenwirkung näher zu konkretisieren und gleichzeitig den Einfluss des Sauerstoffdrucks und die Wirkung schwefelhaltiger 'Schutzstoffe' auf die direkte Strahlenwirkung zu deuten. Wesentliche Fortschritte auf dem Wege zur Lösung dieser Fragen scheinen uns zwei Gruppen von Arbeiten gebracht zu haben, deren eine sich mit dem Stoffwechsel und Wirkungsmechanismus schwefelhaltiger 'Schutzstoffe' (ELDJARN und PIHL, 1956) und deren andere sich mit der Mikrowellenspektroskopie bestrahlter Gewebe und biologisch wichtiger Substanzen (Aminosäuren, Nucleinsäuren und Proteine) befasst (GORDY, ARD und SHIELDS, 1955).

Die chemischen Untersuchungen, die bisher nur für Cystamin und Cysteamin zum Abschluss gebracht sind, ergaben ganz eindeutig, dass diese Stoffe schon in so geringer Menge wirken, dass bei Annahme gleichmässiger Verteilung über alle Zellbestandteile ihre Konzentration viel zu gering ist, um eine Schutzwirkung gegen Strahlung nach dem von vielen Autoren angenommenen Mechanismus des Wettbewerbs um die Reaktionsprodukte des Wassers zu erklären. Es konnte vielmehr gezeigt werden, dass Bindungen mit SH-Gruppen vieler Proteine eingegangen werden, und dass die Schutzwirkung darin zu bestehen scheint, dass die Strahlenenergie zum Wiederauflösen dieser Bindungen verbraucht wird und so eine Beschädigung des Proteins verhindert wird.

Diese Hypothese erhielt durch die ganz unabhängig durchgeführten Untersuchungen der Mikrowellenspektren eine starke Stütze. Sie schliesst ja die, allerdings nach anderen Untersuchungen sehr wahrscheinliche, Annahme ein, dass eine irgendwo im Proteinmolekül auftretende Ionisation oder Energizufuhr im allgemeinen unwirksam bleibt, indem das Loch oder die Energie zur SS-Bindung wandert und diese in gewisser Weise verändert, eventuell löst. Gerade das Auftreten eines solchen Wanderungsvorganges muss aus den Mikrowellenspektren von Aminosäuren und Proteinen, die mit Röntgenstrahlen bestrahlt waren, gefolgert werden. Mittels der angewandten Methode werden Absorptionsfrequenzen im Mikrowellengebiet gemessen, die durch die Resonanz unpaariger

Elektronen in von aussen aufgegebenen Magnetfeldern verursacht werden. Das für uns hier wichtige Ergebnis besteht nun darin, das bei vielen bestrahlten Keratinen immer nur das vorher an reinem Cystin gemessene Cystinspektrum gefunden wurde, es jedoch niemals gelang, die ebenfalls vorher bestimmten Spektren der anderen viel häufiger vorkommenden Aminosäuren zu finden. Es besteht daher Grund zur Annahme, dass alle im Protein erfolgenden durch Ionisation verursachten Störungen immer zum schwefelhaltigen Cystin wandern. Wenn damit auf grund der erwähnten chemischen und physikalischen Untersuchungen eine neue und befriedigendere Deutungsmöglichkeit für den Mechanismus der Wirkung schwefelhaltiger Schutzstoffe gefunden zu sein scheint, so muss in Übereinstimmung mit dem einleitend Ausgeführten davor gewarnt werden, diese Deutung als universell gültig anzusehen. Sie bedarf natürlich erstens noch weiterer Prüfung, und zweitens sind andere Mechanismen dadurch nicht ausgeschlossen. Immerhin bietet die beschriebene Hypothese einige nach unserer Ansicht erhebliche Vorteile, indem sie Schutzwirkung auch gegen 'direkte' Strahlungseffekte in den Kreis der erklärbaren Phänomene einbezieht. Insbesondere macht sie die Erscheinung des 'Hyperschutzes' an Bakteriophagen (DOERMAN, 1952) verständlich, die darin besteht, dass die Röntgenstrahlenwirkung auf T2H-Phagen durch Zugabe von Cystein noch um die Hälfte vermindert werden kann, selbst wenn die Phagen in so konzentrierter Bouillon bestrahlt werden, dass Verwendung noch höherer Konzentration keine weitere Verminderung der Strahlenwirkung mehr bringt, die 'indirekte' Strahlenwirkung also nach Ansicht vieler Autoren ausgeschlossen ist. Dieser wichtige, aber nicht sehr oft erwähnte Befund war ja nach dem Mechanismus der Konkurrenz um die durch Strahlung erzeugten Reaktionsprodukte des Wassers nicht ohne zusätzliche Hilfsannahmen zu verstehen.

Die für die Strahlenbiologie wichtigen Befunde der Mikrowellenspektroskopie gehen jedoch noch wesentlich weiter und bieten auch neue Deutungsmöglichkeiten für den Einfluss des Sauerstoffs auf Strahlenwirkungen. Es konnte von den erwähnten Autoren nämlich gezeigt werden, dass in Proteinen und auch in Nucleinsäuren nach Bestrahlung Resonanzen auftreten, die über Tage bestehen bleiben, wenn Röntgenbestrahlung und Messung unter Abschluss von Sauerstoff vorgenommen wurden. Nach Zutritt von Sauerstoff klingen die Resonanzen im allgemeinen schnell ab. Nur in wenigen Fällen wurde nach Sauerstoffeinwirkung nicht nur das Abklingen der zuerst aufgetretenen, sondern auch die Bildung neuer, in Luft stabiler Resonanzen beobachtet. Da die beobachteten Resonanzen von unpaarigen Elektronen herrühren und diese in den fraglichen Stoffen auf das Vorliegen von Radikalen schliessen lassen, kommt man zu der Vorstellung, dass die 'direkte' Strahlenwirkung über den Mechanismus der Bildung langlebiger Radikale verläuft, die bei Anwesenheit von Sauerstoff weiter reagieren und dann mit grösserer Wahrscheinlichkeit zu biologisch wichtigen Veränderungen führen.

Es erscheint verfrüht, an die mitgeteilten Befunde jetzt schon detaillierte Spekulationen zu knüpfen. Wir möchten nur darauf hinweisen, dass man damit eine Reihe neuer Vorstellungen über das Wesen des 'direkten Treffers', über den Wirkungsmechanismus mancher Schutzstoffe und über den Einfluss des Sauerstoffdrucks auf strahlenbiologische Reaktionen, sowie schliesslich auch über viele bisher schwer deutbare 'Nachwirkungseffekte' erhält, die zu interes-

santen Ansatzpunkten für weitere Arbeit, speziell auch auf dem Gebiete der Strahlengenetik, führen.

Es ist uns eine angenehme Pflicht, der Knut und Alice Wallenberg-Stiftung und dem Forschungsrat für Landwirtschaft für Unterstützung unserer Arbeit zu danken.

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A. Håkansson: Chromosome number and meiosis in *Salix* (*grandifolia* × *gracilistyla*) × *S.* (*silesiaca* × *aegyptiaca*).

This quadruple hybrid which the late Professor Heribert Nilsson had noted as »promising», does not appear to have been used by him to any higher degree in further crosses. The female parent was a hybrid between two diploid species, *S. grandifolia* and *S. gracilistyla*, the male parent being a hybrid between the diploid *S. silesiaca* and the tetraploid *S. aegyptiaca*. The latter hybrid is triploid and its meiosis shows the occasional production of unreduced (triploid) pollen grains (unpublished). The original number of shrubs produced in the cross seems to have been fourteen, all were female. Six shrubs were now available for a cytological investigation.

Chromosome counts in root tips and at meiosis showed that all shrubs investigated were tetraploid ($2n=76$, $n=38$). Meiosis was studied in three of them. At first metaphase a regular plate is formed containing, in several cases observed, only bivalents. Other plates proved the occasional formation of one or two quadrivalents or trivalents and a few univalents. The plate in Fig. 1 (to the left)

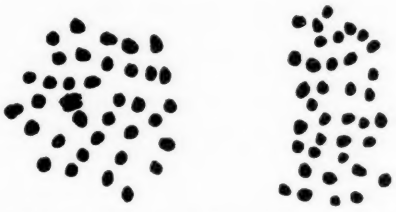

Fig. 1. Plates of first metaphase (left) and second metaphase (right).

has $1_{IV}+36_{II}$. First anaphase rather frequently showed lagging, but was on the whole regular and elimination of chromosomes was rarely observed. Meiosis in this hybrid is somewhat delayed, and as in other Salices with delayed meiosis the second spindle often takes an oblique or nearly transverse position in the lower dyad cell. This unusual direction of the spindle is no doubt a consequence of the increased diameter of the megaspore mother cell and the lower dyad cell when meiosis has been delayed. The normal direction of the spindle in *Salix* is longitudinal in the lower, oblique in the upper dyad cell, which is

broader. In the lower cell the plate is sometimes large and the chromosomes may be counted (Fig. 1, to the right). The embryo sac is formed by the innermost megaspore.

Clearly, fertilizations had been performed with unreduced, triploid male gametes. It is peculiar that the progeny of the cross seemed to consist exclusively of tetraploids, all shrubs being vigorous and with a rather uniform appearance. Presumably the pollen tubes with aneuploid gametes are not functional; diploid tubes (38 chromosomes) are expected to function but are certainly more rare. An alternative, less probable explanation could be selection on the zygotic level, zygotes with aneuploid numbers being formed though aborting during seed development and only tetraploids surviving.

In the crosses of NILSSON several new polyploid forms have appeared, in most cases accompanied by a larger or smaller number of plants with »expected» chromosome numbers. In some cases there were only new polyploids, but then the progeny consisted of merely a few shrubs (HÅKANSSON, 1955). In the case now described, the tetraploid progeny seemed unexpectedly large.

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R. LAMM: Localization of *Gp* and *R* in *Pisum*.

In F_1, between the structural types Extra Rapid N and Thibet 7, the first metaphase of meiosis contains five bivalents and one non-ring-forming configuration of four chromosomes (LAMM, 1951). The gametic abortion is higher than in simple translocation heterozygotes. These observations confirm the results of cytological investigations showing that Extra Rapid N and Thibet 7 are reciprocal translocations both involving chromosomes 1 and 3 but with different positions of the breaks.

In progenies from translocation heterozygotes between the standard untranslocated type and three different translocation homozygotes, the following linkage relations between points of interchange and marker genes have been found:

Structural type	T-Gene	Linkage value	Reference
Thibet 7	T(1—3)a—*B*	1,0 ± 1,06 %	LAMM, 1951
» »	» —*St*	27,0 ± 3,30 »	» »
» »	» —*Gp*	16,9 ± 3,28 »	» unpubl.
» »	» —*R*	5,5 ± 0,75 »	» 1951
Extra Rapid N	T(1—3)b—*B*	17,5 ± 1,72 »	» 1948
» »	» —*St*	8,0 ± 0,96 »	» »
» »	» —*Gp*	6,5 ± 3,32 »	» 1949
» »	» —*R*	46,2 ± 3,99 »	NILSSON, 1950
Winge	T(1—4) —*Gp*	28,3 ± 4,28 »	LAMM, unpubl.
»	» —*R*	1,4 ± 1,38 »	» »

Translocations involving chromosome 3 give linkage with the genes *B* and *St*. In addition to Thibet 7 and Extra Rapid N this has been shown in N III [T(3—5)] by NILSSON (1950) and in Merton 1 [T(3—4)] by LAMM (unpublished). *B*—*St* is thus a segment of chromosome 3. The linkage results given above show that chromosome 1 contains the genes *Gp* and *R*, although in Extra Rapid N the T(1—3)b—*R* linkage is non-significant. As to chromosome 1 and 3 the following order of points of interchange and genes has been found:

chromosome 1 —T(1—3)b—*Gp*—T(1—3)a—*R*—
» 3 —T(1—3)b—*St*—*B*—T(1—3)a—

If the centromere of chromosome 1 were situated between T(1—3)b and *Gp* the crossing-over values between *Gp* and *R* would be unaffected in the Extra Rapid N but reduced in the Thibet 7 translocation heterozygote (*cf.* LAMM, 1948). This is a hypothetical explanation of the doubtful linkage between T(1—3)b and *R*. It should also be mentioned that the structural type Winge was chosen for the *Gp*—*R* investigation because of the long interstitial, crossing-over, reducing segment typical of this type (*cf.* SUTTON, 1935).

According to LAMPRECHT's work (1948) *Gp* and *R* are situated in different chromosomes of the standard structural types. The present investigation constitutes strong evidence against this assumption.

Finally, it should be mentioned that the genetical studies of the progenies from the Thibet 7 translocation heterozygote suffer from one unsolved complication, *viz.*, the linkage between T(1—3)a and *I*. This gene is neither situated in chromosome 1 nor in chromosome 3. This unexpected behaviour may perhaps be due to accidental circumstances or to so-called affinity. A special investigation of this question has been initiated, and a full report of the *Gp*—*R* linkage will be given when results of the affinity and other tests are available.

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